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(54) Title: RECEPTOR FOR A BACILLUS THURINGIENSIS TOXIN

(57) Abstract

The cDNA that encodes a glycoprotein receptor from the tobacco hornworm which binds a *Bacillus thuringiensis* toxin has been obtained and sequenced. The availability of this cDNA permits the retrieval of DNAs encoding homologous receptors in other insects and organisms as well as the design of assays for the cytotoxicity and binding affinity of potential pesticides and the development of methods to manipulate natural and/or introduced homologous receptors and, thus, to destroy target cells, tissues and/or organisms.

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RECEPTOR FOR A BACILLUS THURINGIENSIS TOXIN

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Technical Field

The invention relates to receptors that bind toxins from *Bacillus thuringiensis* and thus to pesticides and pest resistance. More particularly, the invention concerns recombinantly produced receptors that bind BT toxin and to their use in assays for improved pesticides, as well as in mediation of cell and tissue destruction, dissociation, dispersion, cell-to-cell association, and changes in morphology.

Background Art

It has long been recognized that the bacterium *Bacillus thuringiensis* (BT) produces bactericidal proteins that are toxic to a limited range of insects, mostly in the orders Lepidoptera, Coleoptera and Diptera. Advantage has been taken of these toxins in controlling pests, mostly by applying bacteria to plants or transforming plants themselves so that they generate the toxins by virtue of their transgenic character. The toxins themselves are glycoprotein products of the *cry* gene as described by Höfte, H. *et al. Microbiol Rev* (1989) 53:242. It has been established that the toxins function in the brush border of the insect midgut epithelial cells as described by Gill, S.S. *et al. Annu Rev Entomol* (1992) 37:615. Specific binding of BT toxins to midgut brush border membrane vesicles has been reported by Hofmann, C. *et al. Proc Natl Acad Sci USA* (1988) 85:7844; Van Rie, J. *et al. Eur J Biochem* (1989) 186:239; and Van Rie, J. *et al. Appl Environ Microbiol* (1990) 56:1378.

Presumably, the toxins generated by BT exert their effects by some kind of interaction with receptors in the midgut. The purification of a particular receptor from *Manduca sexta* was reported by the present inventors in an article by Vadlamudi, R.K. *et al. J Biol Chem* (1993) 268:12334. In this report, the receptor protein was isolated by immunoprecipitating toxin-binding protein complexes with toxin-specific antisera and separating the complexes by SDS-PAGE followed by electroelution. However, to date, there has been no structural information concerning any insect receptor which binds BT toxin, nor have, to applicants' knowledge, any genes encoding these receptors been recovered.

Disclosure of the Invention

The present invention is based, in part, on the isolation and characterization of a receptor that is bound by members of the BT-toxin family of insecticidal proteins, hereinafter the BT-R_1 protein. The present invention is further based on the isolation and characterization of a nucleic acid molecule that encodes the BT-toxin receptor, hereinafter *BT-R1* gene. Based on these observations, the present invention provides compositions and methods for use in identifying agents that bind to the BT-R_1 protein as a means for identifying insecticidal agent and for identifying other members of the BT-R_1 family of proteins.

Brief Description of the Drawings

Figure 1 show the nucleotide sequence and deduced amino acid sequence of cDNA encoding the BT-R_1 protein from *M. sexta*.

Figure 2 (panels a and b) shows a comparison of amino acid sequences of cadherin motifs (BTRcad-1 to 11) in BT-R_1 to those of other cadherins.

Figure 3 shows a block diagram of the cadherin-like structure of BT-R_1.

Figure 4 shows the clone characterization of the BamHI-SacI fragment of BT-R_1. LM is HindIII cut Lambda marker; UP is the uncut plasmid clone; NP is NsiI cut plasmid; XP is XhoI cut plasmid; BSP is BamHI and SacI cut plasmid showing

the cloned fragment from BT-R_1; RM is mRNA size marker; and RT1 and RT2 are transcribed mRNAs from the cloned BT-R_1 fragment.

Figure 5 illustrates the detection of protein expression from the plasmid containing the Bam-Sac fragment of BT-R_1 using ^{35}S-methionine as a tag. LCR is a luciferase control mRNA to show that the rabbit reticulocyte lysates are functional; RR1 and RR2 are expression products of the Bam-Sac fragment of BT-R_1 produced in rabbit reticulocytes from mRNA; LCT is a luciferase control plasmid to show that the transcription/translation kit is functional; and TT1 and TT2 are expression products of the Bam-Sac fragment of BT-R_1 produced in a transcription/translation kit.

Figure 6 shows a radio-blot of the Bam-Sac fragment of BT-R_1 with ^{125}I-labeled Cry1Ab. BBMV is the brush border membrane vesicles from the midgut of *M. sexta* containing the wild-type BT-R_1 receptor protein; RBK is a rabbit reticulocyte blank; RR1 and RR2 are the expression products of the Bam-Sac fragment of BT-R_1 produced in rabbit reticulocytes from mRNA; TBK is a transcription/translation kit blank; TT1 and TT2 are expression products of the Bam-Sac fragment of BT-R_1 produced in a transcription/translation kit. The arrows point to two of the bands.

Figure 7 shows the presence of a BT-R_1 homologue in Pink Bollworm and European Corn Borer identified using toxin binding similar to that used to identify the original BT-R_1 clone.

Figure 8 shows the binding of Cry1Ab to fragments of the BT-R_1 protein.

Modes of Carrying Out the Invention

I. General Description

The present invention is based, in part, on the isolation and characterization of a novel protein expressed in the midgut of *Manduca sexta* that binds to members of the BT-toxin family of proteins, hereinafter the BT-R_1 protein. The present invention specifically provides purified BT-R_1, the amino acid sequence of BT-R_1, as well as nucleotide sequences that encode BT-R_1. The BT-R_1 protein and nucleic acid molecules can serve as targets in identifying insecticidal agents.

II. Specific Embodiments

A. BT-R_1 Protein

Prior to the present invention, although members of the BT-toxin family of protein were known, no one had identified the receptor that is bound by these toxin proteins. The present invention provides, in part, the amino acid sequences of a BT-toxin receptor that is expressed in the midgut of *Maduca sexta*.

In one embodiment, the present invention provides the ability to isolate or produce a previously unknown protein by using known purification methods, the cloned nucleic acid molecules herein described or by synthesizing a protein having the amino acid sequence herein disclosed.

As used herein, BT-R_1 refers to a protein that has the amino acid sequence of BT-R_1 provided in Figure 1, as well as allelic variants of the BT-R_1 sequence, and conservative substitutions mutants of the BT-R_1 sequence that have BT-R_1 activity. BT-R_1 is comprised of a single subunit, has a molecular weight of 210 kD, and has the amino acid sequence provided in Figure 1. A prediction of the structure of BT-R_1 is provided in Figure 3.

The BT-R_1 protein of the present invention includes the specifically identified and characterized variant herein described, as well as allelic variants, conservative substitution variants and homologues (Figure 7) that can be isolated/generated and characterized without undue experimentation following the methods outlined below. For the sake of convenience, all BT-R_1 proteins will be collectively referred to as the BT-R_1 proteins, the BT-R_1 proteins of the present invention or BT-R_1.

The term "BT-R_1" includes all naturally occurring allelic variants of the *Manduca sexta* BT-R_1 protein provided in Figure 1. In general, naturally occurring allelic variants of *Manduca sexta* BT-R_1 will share significant homology, at least 75 %, and generally at least 90%, to the BT-R_1 amino acid sequence provided in Seq. ID No:2. Allelic variants, though possessing a slightly different amino acid sequence than Seq. ID No:2, will be expressed as a transmembrane protein in the digestive tract of an insect or other organism. Typically, allelic variants of the BT-R_1 protein will

contain conservative amino acid substitutions from the BT-R_1 sequence herein described or will contain a substitution of an amino acid from a corresponding position in a BT-R_1 homologue (a BT-R_1 protein isolated from an organism other than *Manduca sexta*).

One class of BT-R_1 allelic variants will be proteins that share a high degree of homology with at least a small region of the amino acid sequence provided in Seq. ID No:__, but may further contain a radical departure from the sequence, such as a non-conservative substitution, truncation, insertion or frame shift. Such alleles are termed mutant alleles of BT-R_1 and represent proteins that typically do not perform the same biological functions as does the BT-R_1 variant of Seq. ID No:2.

The BT-R_1 proteins of the present invention are preferably in isolated form. As used herein, a protein is said to be isolated when physical, mechanical or chemical methods are employed to remove the BT-R_1 protein from cellular constituents that are normally associated with the protein. A skilled artisan can readily employ standard purification methods to obtain an isolated BT-R_1 protein. The nature and degree of isolation will depend on the intended use.

The cloning of the BT-R_1 encoding nucleic acid molecule makes it possible to generate defined fragments of the BT-R_1 proteins of the present invention. As discussed below, fragments of BT-R_1 are particularly useful in: generating domain specific antibodies; identifying agents that bind to toxin binding domain on BT-R_1; identifying toxin-binding structures; identifying cellular factors that bind to BT-R_1; isolating homologues or other allelic forms of BT-R_1; and studying the mode of action of BT-toxins.

Fragments of the BT-R_1 proteins can be generated using standard peptide synthesis technology and the amino acid sequence of *Manduca sexta* BT-R_1 disclosed herein. Alternatively, as illustrated in Example 5, recombinant methods can be used to generate nucleic acid molecules that encode a fragment of the BT-R_1 protein. Fragments of the BT-R_1 protein subunits that contain particularly interesting structures can be identified using art-known methods such as by using an immunogenicity plot, Chou-Fasman plot, Garnier-Robson plot, Kyte-Doolittle plot,

Eisenberg plot, Karplus-Schultz plot or Jameson-Wolf plot of the BT-R_1 protein. Fragments containing such residues are particularly useful in generating domain specific anti-BT-R_1 antibodies or in identifying cellular factors that bind to BT-R_1. One particular fragment that is preferred for use in identifying insecticidal agents is a soluble fragment of BT-R_1 that can bind to a member of the BT family of toxins. In Example 5, a fragment of BT-R_1 that binds to a BT-toxin is disclosed.

As described below, members of the BT-R_1 family of proteins can be used for, but are not limited to: 1) a target to identify agents that bind to BT-R_1, 2) a target or bait to identify and isolate binding partners and cellular factors that bind to BT-R_1, 3) an assay target to identify BT-R_1 and other receptor-mediated activity, and 4) a marker of cells that express a member of the BT-R_1 family of proteins.

B. Anti-BT-R_1 Antibodies

The present invention further provides antibodies that bind BT-R_1. The most preferred antibodies will selectively bind to BT-R_1 and will not bind (or will only bind weakly) to non-BT-R_1 proteins. Anti- BT-R_1 antibodies that are especially contemplated include monoclonal and polyclonal antibodies as well as fragments containing the antigen binding domain and/or one or more complement determining regions (CDRs) of these antibodies.

Antibodies are generally prepared by immunizing a suitable mammalian host using a BT-R_1 protein (synthetic or isolated), or fragment, in isolated or immunoconjugated form (Harlow, Antibodies, Cold Spring Harbor Press, NY (1989)). Regions of the BT-R_1 protein that show immunogenic structure can readily be identified using art-known methods. Other important regions and domains can readily be identified using protein analytical and comparative methods known in the art, such as Chou-Fasman, Garnier-Robson, Kyte-Doolittle, Eisenberg, Karplus-Schultz or Jameson-Wolf analysis. Fragments containing these residues are particularly suited in generating specific classes of anti-BT-R_1 antibodies. Particularly useful fragments include, but are not limited to, the BT-toxin binding domain of BT-R_1 identified in Example 5.

Methods for preparing a protein for use as an immunogen and for preparing immunogenic conjugates of a protein with a carrier such as BSA, KLH, or other carrier proteins are well known in the art. In some circumstances, direct conjugation with reagents such as carbodiimide may be used; in other instances linking reagents like those supplied by Pierce Chemical Co., Rockford, IL, may be effective.

Administration of a BT-R_1 immunogen is conducted generally by injection over a suitable time period in combination with a suitable adjuvant, as is generally understood in the art. During the immunization schedule, titers of antibodies can be taken to determine adequacy of antibody formation.

Although the polyclonal antisera produced in this way may be satisfactory for some applications, for many other applications, monoclonal antibody preparations are preferred. Immortalized cell lines which secrete a desired monoclonal antibody may be prepared using the standard method of Kohler and Milstein or modifications which effect immortalization of lymphocytes or spleen cells, as is generally known. The immortalized cell lines secreting the desired antibodies are screened by immunoassay in which the antigen is the BT-R_1 protein or BT-R_1 fragment. When the appropriate immortalized cell culture secreting the desired antibody is identified, the cells can be cultured either *in vitro* or by production in ascites fluid.

The desired monoclonal antibodies are then recovered from the culture supernatant or from the ascites supernatant. Fragments of the monoclonals or the polyclonal antisera which contain the immunologically significant portion can be used as antagonists, as well as the intact antibodies. Use of immunologically reactive fragments, such as the Fab, Fab', of $F(ab')_2$ fragments is often preferable, especially in a therapeutic context, as these fragments are generally less immunogenic than the whole immunoglobulin.

The antibodies or fragments may also be produced, using current technology, by recombinant means. Regions that bind specifically to the desired regions of the BT-R_1 protein can also be produced in the context of chimeric or CDR grafted antibodies of multiple species origin.

As described below, anti-BT-R_1 antibodies are useful as modulators of BT-R_1 activity, are useful in *in vitro* and *in vivo* antibody based assays methods for detecting BT-R_1 expression/activity, in generating toxin conjugates, for purifying homologues of *Manduca sexta* BT-R_1, in generating anti-ideotypic antibodies that mimic the BT-R_1 protein and in identifying competitive inhibitors of BT-toxin/BT-R_1 interactions.

C. BT-R_1 Encoding Nucleic Acid Molecules

As described above, the present invention is based, in part, on isolating nucleic acid molecules from *Manduca sexta* that encode BT-R_1. Accordingly, the present invention further provides nucleic acid molecules that encode the BT-R_1 protein, as herein defined, preferably in isolated form. For convenience, all BT-R_1 encoding nucleic acid molecules will be referred to as BT-R_1 encoding nucleic acid molecules, the *BT-R1* genes, or *BT-R1*. The nucleotide sequence of the *Manduca sexta* nucleic acid molecule that encodes one allelic form of BT-R_1 is provided in Figure 1.

As used herein, a "nucleic acid molecule" is defined as an RNA or DNA molecule that encodes a peptide as defined above, or is complementary to a nucleic acid sequence encoding such peptides. Particularly preferred nucleic acid molecules will have a nucleotide sequence identical to or complementary to the *Manduca sexta* DNA sequences herein disclosed. Specifically contemplated are genomic DNA, cDNAs, synthetically prepared DNAs, and antisense molecules, as well as nucleic acids based on an alternative backbone or including alternative bases, whether derived from natural sources or synthesized. A skilled artisan can readily obtain these classes of nucleic acid molecules using the herein described *BT-R1* sequences. However, such nucleic acid molecules, are defined further as being novel and unobvious over any prior art nucleic acid molecules encoding non-BT-R_1 proteins. For example, the *BT-R1* sequences of the present invention specifically excludes previously identified nucleic acid molecules that share only partial homology to *BT-R1*. Such excluded sequences include identified members of the cadhedrin family of proteins.

As used herein, a nucleic acid molecule is said to be "isolated" when the nucleic acid molecule is substantially separated from contaminant nucleic acid molecules that encode polypeptides other than BT-R_1. A skilled artisan can readily employ nucleic acid isolation procedures to obtain an isolated BT-R_1 encoding nucleic acid molecule.

The present invention further provides fragments of the BT-R_1 encoding nucleic acid molecules of the present invention. As used herein, a fragment of a BT-R_1 encoding nucleic acid molecule refers to a small portion of the entire *BT-R1* sequence. The size of the fragment will be determined by its intended use. For example, if the fragment is chosen so as to encode the toxin binding domain of BT-R_1 identified in Example 5, then the fragment will need to be large enough to encode the toxin binding domain of the BT-R_1 protein. If the fragment is to be used as a nucleic acid probe or PCR primer, then the fragment length is chosen so as to obtain a relatively small number of false positives during probing/priming. Fragments of the *Manduca sexta BT-R1* gene that are particularly useful as selective hybridization probes or PCR primers can be readily identified from the entire *BT-R1* sequence using art-known methods.

Another class of fragments of BT-R_1 encoding nucleic acid molecules are the expression control sequence found upstream and downstream from the BT-R_1 encoding region found in genomic clones of the *BT-R1* gene. Specifically, tissue and developmental specific expression control elements can be identified as being 5' to the BT-R_1 encoding region found in genomic clones of the *BT-R1* gene. Such expression control sequence are useful in generating expression vectors for expressing genes in the digestive tract of a transgenic organism. As described in more detail below, a skilled artisan can readily use the *BT-R1* cDNA sequence herein described to isolate and identify genomic *BT-R1* sequences and the expression control elements found in the *BT-R1* gene.

Fragments of the BT-R_1 encoding nucleic acid molecules of the present invention (i.e., synthetic oligonucleotides) that are used as probes or specific primers for the polymerase chain reaction (PCR), or to synthesize gene sequences encoding BT-R_1 proteins, can easily be synthesized by chemical techniques, for example, the

phosphotriester method of Matteucci, *et al.*, *J Am Chem Soc* (1981) 103:3185-3191, or using automated synthesis methods. In addition, larger DNA segments can readily be prepared by well known methods, such as synthesis of a group of oligonucleotides that define various modular segments of the *BT-R1* gene, followed by ligation of oligonucleotides to build the complete modified *BT-R1* gene.

The BT-R_1 encoding nucleic acid molecules of the present invention may further be modified so as to contain a detectable label for diagnostic and probe purposes. As described above, such probes can be used to identify nucleic acid molecules encoding other allelic variants or homologues of the BT-R_1 proteins and as described below, such probes can be used to identify the presence of a BT-R_1 protein as a means for identifying cells that express a BT-R_1 protein. A variety of such labels are known in the art and can readily be employed with the BT-R_1 encoding molecules herein described. Suitable labels include, but are not limited to, biotin, radiolabeled nucleotides, biotin, and the like. A skilled artisan can employ any of the art-known labels to obtain a labeled BT-R_1 encoding nucleic acid molecule.

D. Isolation of Other BT-R_1 Encoding Nucleic Acid Molecules

The identification of the BT-R_1 protein from *Manduca sexta* and the corresponding encoding nucleic acid molecules, has made possible the identification of and isolation of: 1) BT-R_1 proteins from organisms other than *Manduca sexta*, hereinafter referred to collectively as BT-R_1 homologues, 2) other allelic and mutant forms of the *Manduca sexta* BT-R_1 protein (described above), and 3) the corresponding genomic DNA that contains the *BT-R1* gene. The most preferred source of BT-R_1 homologues are insects, the most preferred being members of the Lepidopteran, Coleopteran and Dipteran orders of insects. Evidence of the existence of BT-R_1 homologues is provided in Figure 7.

Essentially, a skilled artisan can readily use the amino acid sequence of the *Manduca sexta* BT-R_1 protein to generate antibody probes to screen expression libraries prepared from cells and organisms. Typically, polyclonal antiserum from mammals such as rabbits immunized with the purified protein (as described above) or

monoclonal antibodies can be used to probe an expression library, prepared from a target organism, to obtain the appropriate coding sequence for a BT-R_1 homologue. The cloned cDNA sequence can be expressed as a fusion protein, expressed directly using its own control sequences, or expressed by constructing an expression cassette using control sequences appropriate to the particular host used for expression of the enzyme.

Alternatively, a portion of the BT-R_1 encoding sequence herein described can be synthesized and used as a probe to retrieve DNA encoding a member of the BT-R_1 family of proteins from organisms other than *Manduca sexta*, allelic variants of the *Manduca sexta* BT-R_1 protein herein described, and genomic sequence containing the *BT-R1* gene. Oligomers containing approximately 18-20 nucleotides (encoding about a 6-7 amino acid stretch) are prepared and used to screen genomic DNA or cDNA libraries to obtain hybridization under stringent conditions or conditions of sufficient stringency to eliminate an undue level of false positives.

Additionally, pairs of oligonucleotide primers can be prepared for use in a polymerase chain reaction (PCR) to selectively amplify/clone a BT-R_1-encoding nucleic acid molecule, or fragment thereof. A PCR denature/anneal/extend cycle for using such PCR primers is well known in the art and can readily be adapted for use in isolating other BT-R_1 encoding nucleic acid molecules. Regions of the *Manduca sexta BT-R1* gene that are particularly well suited for use as a probe or as primers can be readily identified by one skilled in the art.

Non-*Manduca sexta* homologues of *BT-R1*, naturally occurring allelic variants of the *Manduca sexta BT-R1* gene and genomic *BT-R1* sequences will share a high degree of homology to the *Manduca sexta BT-R1* sequence herein described. In general, such nucleic acid molecules will hybridize to the *Manduca sexta BT-R1* sequence under high stringency. Such sequences will typically contain at least 70% homology, preferably at least 80%, most preferably at least 90% homology to the *Manduca sexta BT-R1* sequence of Seq. ID No:1.

In general, nucleic acid molecules that encode homologues of the *Manduca sexta* BT-R_1 protein will hybridize to the *Manduca sexta BT-R1* sequence under stringent conditions. "Stringent conditions" are those that (1) employ low ionic

strength and high temperature for washing, for example, 0.015M NaCl/0.0015M sodium titrate/0.1% SDS at 50°C., or (2) employ during hybridization a denaturing agent such as formamide, for example, 50% (vol/vol) formamide with 0.1% bovine serum albumin/0.1% Ficoll/0.1% polyvinylpyrrolidone/50 mM sodium phosphate buffer at pH 6.5 with 750 mM NaCl, 75 mM sodium citrate at 42°C. Another example is use of 50% formamide, 5 x SSC (0.75M NaCl, 0.075 M sodium citrate), 50 mM sodium phosphate (pH 6.8), 0.1% sodium pyrophosphate, 5 x Denhardt's solution, sonicated salmon sperm DNA (50 μg/ml), 0.1% SDS, and 10% dextran sulfate at 42°C., with washes at 42°C. in 0.2 x SSC and 0.1% SDS. A skilled artisan can readily determine and vary the stringency conditions appropriately to obtain a clear and detectable hybridization signal.

The presence of similar receptors in noninsect organisms as well as other insects besides those harboring BT-R_1 is supported by the sequence similarity of the BT-R_1 protein to that of the various members of the cadherin superfamily of proteins, which are membrane glycoproteins believed to mediate calcium-dependent cell aggregation and sorting. See, for example, Takeichi, M. *Science* (1991) 251:1451; and Takeichi, M. *N Rev Biochem* (1990) 59:237.

Included in this superfamily are desmoglien, desmocollins, the *Drosophila fat* tumor suppressor, *Manduca sexta* intestinal peptide transport protein and T-cadherin. All of these proteins share common extracellular motifs although their cytoplasmic domains differ. Goodwin, L. *et al. Biochem Biophys Res Commun* (1990) 173:1224; Holton, J.L. *et al. J Cell Sci* (1990) 97:239; Bestal, D.J. *J Cell Biol* (1992) 119:451; Mahoney, P.A. *et al. Cell* (1991) 853; Dantzig, A.H. *et al. Science* (1994) 264:430; and Sano, K. *et al. EMBO J* (1993) 12:2249. Inclusion of BT-R_1 in the cadherin superfamily is further supported by the report that EDTA decreases the binding of *CryIAb* toxin of BT to the 210 kD receptor of *M. sexta* (Martinez-Ramirez, A.C. *et al. Biochm Biophys Res Commun* (1994) 201:782).

It is noted below that the amino acid sequence of BT-R_1 reveals that a calcium-binding motif is present. This is consistent with the possibility that cells having receptors to bind toxin may themselves survive although they render the tissues in

which they are included permeable to solutes and thus effect disintegration of the tissue. Such a mechanism is proposed for the death of insects that ingest the toxin via the epithelial cells in their midgut by Knowles, B.H. *et al. Biochim Biophys Acta* (1987) 924:509. Such a mechanism is also supported in part by the results set forth in Example 4 hereinbelow which indicate that the effect of the toxin on embryonic 293 cells modified to express the receptor at their surface is reversible.

E. rDNA Molecules Containing a BT-R_1 Encoding Nucleic Acid Molecule

The present invention further provides recombinant DNA molecules (rDNAs) that contain a BT-R_1 encoding sequences as herein described, or a fragment thereof, such as a soluble fragment of BT-R_1 that contains the BT-toxin binding site. As used herein, a rDNA molecule is a DNA molecule that has been subjected to molecular manipulation *in vitro*. Methods for generating rDNA molecules are well known in the art, for example, see Sambrook *et al., Molecular Cloning* (1989). In the preferred rDNA molecules of the present invention, a BT-R_1 encoding DNA sequence that encodes a BT-R_1 protein or a fragment of BT-R_1, is operably linked to one or more expression control sequences and/or vector sequences.

The choice of vector and/or expression control sequences to which the BT-R_1 encoding sequence is operably linked depends directly, as is well known in the art, on the functional properties desired, e.g., protein expression, and the host cell to be transformed. A vector contemplated by the present invention is at least capable of directing the replication or insertion into the host chromosome, and preferably also expression, of the BT-R_1 encoding sequence included in the rDNA molecule.

Expression control elements that are used for regulating the expression of an operably linked protein encoding sequence are known in the art and include, but are not limited to, inducible promoters, constitutive promoters, secretion signals, enhancers, transcription terminators and other regulatory elements. Preferably, an inducible promoter that is readily controlled, such as being responsive to a nutrient in the host cell's medium, is used. Further, for soluble fragments, it may be desirable to use secretion signals to direct the secretion of the BT-R_1 protein, or fragment, out of the cell.

In one embodiment, the vector containing a BT-R_1 encoding nucleic acid molecule will include a prokaryotic replicon, i.e., a DNA sequence having the ability to direct autonomous replication and maintenance of the recombinant DNA molecule intrachromosomally in a prokaryotic host cell, such as a bacterial host cell, transformed therewith. Such replicons are well known in the art. In addition, vectors that include a prokaryotic replicon may also include a gene whose expression confers a detectable marker such as a drug resistance. Typical bacterial drug resistance genes are those that confer resistance to ampicillin or tetracycline.

Vectors that include a prokaryotic replicon can further include a prokaryotic or viral promoter capable of directing the expression (transcription and translation) of the BT-R_1 encoding sequence in a bacterial host cell, such as *E. coli*. A promoter is an expression control element formed by a DNA sequence that permits binding of RNA polymerase and transcription to occur. Promoter sequences compatible with bacterial hosts are typically provided in plasmid vectors containing convenient restriction sites for insertion of a DNA segment of the present invention. Typical of such vector plasmids are pUC8, pUC9, pBR322 and pBR329 available from Biorad Laboratories (Richmond, CA), pPL and pKK223 available from Pharmacia, Piscataway, NJ.

Expression vectors compatible with eukaryotic cells, preferably those compatible with vertebrate cells, can also be used to variant rDNA molecules that contain a BT-R_1 encoding sequence. Eukaryotic cell expression vectors are well known in the art and are available from several commercial sources. Typically, such vectors are provided containing convenient restriction sites for insertion of the desired DNA segment. Typical of such vectors are PSVL and pKSV-10 (Pharmacia), pBPV-1/pML2d (International Biotechnologies, Inc.), pTDT1 (ATCC, #31255), the vector pCDM8 described herein, and the like eukaryotic expression vectors.

Eukaryotic cell expression vectors used to construct the rDNA molecules of the present invention may further include a selectable marker that is effective in an eukaryotic cell, preferably a drug resistance selection marker. A preferred drug resistance marker is the gene whose expression results in neomycin resistance, i.e., the neomycin phosphotransferase (*neo*) gene. Southern *et al.*, *J Mol Anal Genet* (1982)

1:327-341. Alternatively, the selectable marker can be present on a separate plasmid, and the two vectors are introduced by cotransfection of the host cell, and selected by culturing in the presence of the appropriate drug for the selectable marker.

F. Host Cells Containing an Exogenously Supplied BT-R_1 Encoding Nucleic Acid Molecule

The present invention further provides host cells transformed with a nucleic acid molecule that encodes a BT-R_1 protein of the present invention, either the entire BT-R_1 protein or a fragment thereof. The host cell can be either prokaryotic or eukaryotic. Eukaryotic cells useful for expression of a BT-R_1 protein are not limited, so long as the cell line is compatible with cell culture methods and compatible with the propagation of the expression vector and expression of a *BT-R1* gene. Preferred eukaryotic host cells include, but are not limited to, yeast, insect and mammalian cells, the most preferred being cells that do not naturally express a BT-R_1 protein.

Any prokaryotic host can be used to express a BT-R_1-encoding rDNA molecule. The preferred prokaryotic host is *E. coli*.

Transformation of appropriate cell hosts with an rDNA molecule of the present invention is accomplished by well known methods that typically depend on the type of vector used and host system employed. With regard to transformation of prokaryotic host cells, electroporation and salt treatment methods are typically employed, see, for example, Cohen *et al.*, *Proc Acad Sci USA* (1972) 69:2110; and Maniatis *et al.*, Molecular Cloning, A Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY (1982). With regard to transformation of vertebrate cells with vectors containing rDNAs, electroporation, cationic lipid or salt treatment methods are typically employed, see, for example, Graham *et al.*, *Virol* (1973) 52:456; Wigler *et al.*, *Proc Natl Acad Sci USA* (1979) 76:1373-76.

Successfully transformed cells, i.e., cells that contain an rDNA molecule of the present invention, can be identified by well known techniques. For example, cells resulting from the introduction of an rDNA of the present invention can be cloned to produce single colonies. Cells from those colonies can be harvested, lysed and their

DNA content examined for the presence of the rDNA using a method such as that described by Southern, *J Mol Biol* (1975) 98:503, or Berent *et al.*, *Biotech* (1985) 3:208 or the proteins produced from the cell assayed via an immunological method.

G. Production of a BT-R_1 Protein Using an rDNA Molecule

The present invention further provides methods for producing a BT-R_1 protein that uses one of the BT-R_1 encoding nucleic acid molecules herein described. In general terms, the production of a recombinant BT-R_1 protein typically involves the following steps.

First, a nucleic acid molecule is obtained that encodes a BT-R_1 protein or a fragment thereof, such as the nucleic acid molecule depicted in Figure 1. The BT-R_1 encoding nucleic acid molecule is then preferably placed in an operable linkage with suitable control sequences, as described above, to generate an expression unit containing the BT-R_1 encoding sequence. The expression unit is used to transform a suitable host and the transformed host is cultured under conditions that allow the production of the BT-R_1 protein. Optionally the BT-R_1 protein is isolated from the medium or from the cells; recovery and purification of the protein may not be necessary in some instances where some impurities may be tolerated.

Each of the foregoing steps can be done in a variety of ways. For example, the desired coding sequences may be obtained from genomic fragments and used directly in an appropriate host. The construction of expression vectors that are operable in a variety of hosts is accomplished using an appropriate combination of replicons and control sequences. The control sequences, expression vectors, and transformation methods are dependent on the type of host cell used to express the gene and were discussed in detail earlier. Suitable restriction sites can, if not normally available, be added to the ends of the coding sequence so as to provide an excisable gene to insert into these vectors. A skilled artisan can readily adapt any host/expression system known in the art for use with BT-R_1 encoding sequences to produce a BT-R_1 protein.

H. Identification of Agents and Cellular Constituents that Bind to a BT-R_1 Protein

Another embodiment of the present invention provides methods for identifying agents and cellular constituents that bind to BT-R_1. Specifically, agents and cellular constituents that bind to BT-R_1 can be identified by: 1) the ability of the agent/constituent to bind to BT-R_1, 2) the ability to block BT-toxin binding to BT-R_1, and/or 3) the ability to kill BT-R_1 expressing cells. Activity assays for BT-R_1 activity and binding and competitive assays using a BT-R_1 protein are suitable for use in high through-put screening methods, particularly using a soluble fragment of BT-R_1 that contains the BT-toxin binding domain, such as that disclosed in Example 5.

In detail, in one embodiment, BT-R_1 is mixed with an agent or cellular extract. After mixing under conditions that allow association of BT-R_1 with the agent or component of the extract, the mixture is analyzed to determine if the agent/component bound to the BT-R_1. Binding agents/components are identified as being able to bind to BT-R_1. Alternatively or consecutively, BT-R_1 activity can be directly assessed as a means for identifying agonists and antagonists of BT-R_1 activity.

Alternatively, targets that are bound by a BT-R_1 protein can be identified using a yeast two-hybrid system or using a binding-capture assay. In the yeast two hybrid system, an expression unit encoding a fusion protein made up of one subunit of a two subunit transcription factor and the BT-R_1 protein is introduced and expressed in a yeast cell. The cell is further modified to contain 1) an expression unit encoding a detectable marker whose expression requires the two subunit transcription factor for expression and 2) an expression unit that encodes a fusion protein made up of the second subunit of the transcription factor and a cloned segment of DNA. If the cloned segment of DNA encodes a protein that binds to the BT-R_1 protein, the expression results in the interaction of the BT-R_1 and the encoded protein. This brings the two subunits of the transcription factor into binding proximity, allowing reconstitution of the transcription factor. This results in the expression of the detectable marker. The yeast two hybrid system is particularly useful in screening a library of cDNA encoding segments for cellular binding partners of BT-R_1.

The BT-R_1 protein used in the above assays can be: an isolated and fully characterized protein, a fragment of a BT-R_1 protein (such as a soluble fragment containing the BT-toxin binding site), a cell that has been altered to express a BT-R_1 protein/fragment or a fraction of a cell that has been altered to express a BT-R_1 protein/fragment. Further, the BT-R_1 protein can be the entire BT-R_1 protein or a defined fragment of the BT-R_1 protein. It will be apparent to one of ordinary skill in the art that so long as the BT-R_1 protein or fragment can be assayed for agent binding, e.g., by a shift in molecular weight or activity, the present assay can be used.

The method used to identify whether an agent/cellular component binds to a BT-R_1 protein will be based primarily on the nature of the BT-R_1 protein used. For example, a gel retardation assay can be used to determine whether an agent binds to BT-R_1 or a fragment thereof. Alternatively, immunodetection and biochip technologies can be adopted for use with the BT-R_1 protein. A skilled artisan can readily employ numerous art-known techniques for determining whether a particular agent binds to a BT-R_1 protein.

Agents and cellular components can be further, or alternatively, tested for the ability to block the binding of a BT-toxin to a BT-R_1 protein/fragment. Alternatively, antibodies to the BT-toxin binding site or other agents that bind to the BT-toxin binding site on the BT-R_1 protein can be used in place of the BT-toxin.

Agents and cellular components can be further tested for the ability to modulate the activity of a BT-R_1 protein using a cell-free assay system or a cellular assay system. As the activities of the BT-R_1 protein become more defined, functional assays based on the identified activity can be employed.

As used herein, an agent is said to antagonize BT-R_1 activity when the agent reduces BT-R_1 activity. The preferred antagonist will selectively antagonize BT-R_1, not affecting any other cellular proteins. Further, the preferred antagonist will reduce BT-R_1 activity by more than 50%, more preferably by more than 90%, most preferably eliminating all BT-R_1 activity.

As used herein, an agent is said to agonize BT-R_1 activity when the agent increases BT-R_1 activity. The preferred agonist will selectively agonize BT-R_1, not

affecting any other cellular proteins. Further, the preferred antagonist will increase BT-R_1 activity by more than 50%, more preferably by more than 90%, most preferably more than doubling BT-R_1 activity.

Agents that are assayed in the above method can be randomly selected or rationally selected or designed. As used herein, an agent is said to be randomly selected when the agent is chosen randomly without considering the specific sequences of the BT-R_1 protein or BT-toxin. An example of randomly selected agents is the use of a chemical library or a peptide combinatorial library, or a growth broth of an organism or plant extract.

As used herein, an agent is said to be rationally selected or designed when the agent is chosen on a nonrandom basis that takes into account the sequence of the target site and/or its conformation in connection with the agent's action. Agents can be rationally selected or rationally designed by utilizing the peptide sequences that make up the BT-R_1 protein and BT-toxin. For example, a rationally selected peptide agent can be a peptide whose amino acid sequence is identical to a fragment of a BT-R_1 protein or BT-toxin.

The agents tested in the methods of the present invention can be, as examples, peptides, small molecules, and vitamin derivatives, as well as carbohydrates. A skilled artisan can readily recognize that there is no limit as to the structural nature of the agents used in the present screening method. One class of agents of the present invention are peptide agents whose amino acid sequences are chosen based on the amino acid sequence of the BT-R_1 protein or BT-toxin. Small peptide agents can serve as competitive inhibitors of BT-R_1 protein activity.

Peptide agents can be prepared using standard solid phase (or solution phase) peptide synthesis methods, as is known in the art. In addition, the DNA encoding these peptides may be synthesized using commercially available oligonucleotide synthesis instrumentation and produced recombinantly using standard recombinant production systems. The production using solid phase peptide synthesis is necessitated if non-gene-encoded amino acids are to be included.

Another class of agents of the present invention are antibodies immunoreactive with critical positions of the BT-R_1 protein. As described above, antibodies are obtained by immunization of suitable mammalian subjects with peptides, containing as antigenic regions, those portions of the BT-R_1 protein intended to be targeted by the antibodies. Critical regions particularly include the BT-toxin binding domain identified in Example 5. Such agents can be used in competitive binding studies to identify second generation BT-R_1 binding agents.

The cellular extracts tested in the methods of the present invention can be, as examples, aqueous extracts of cells or tissues, organic extracts of cells or tissues or partially purified cellular fractions. A skilled artisan can readily recognize that there is no limit as to the source of the cellular extract used in the screening method of the present invention. The preferred source for isolating cellular binding partners of BT-R_1 are cells that express BT-R_1 or cells that are in close proximity to BT-R_1 expressing cells.

An outline of one screening method is as follows. Cells are modified by transfection, retroviral infection, electroporation or other known means, to express a BT-R_1 protein and then cultured under conditions wherein the receptor protein is produced and displayed. If desired, the cells are then recovered from the culture for use in the assay, or the culture itself can be used *per se*.

In the assays, the modified cells are contacted with the candidate toxin and the effect on metabolism or morphology is noted in the presence and absence of the candidate. The effect may be cytotoxic -- i.e., the cells may themselves exhibit one of the indices of cell death, such as reduced thymidine uptake, slower increase in optical density of the culture, reduced exclusion of vital dyes (e.g., trypan blue), increased release of viability markers such as chromium and rubidium, and the like. The differential response between the toxin-treated cells and the cells absent the toxin is then noted. The strength of the toxin can be assessed by noting the strength of the response.

These assays may be conducted directly as described above or competitively with known toxins. For example, one approach might be to measure the diminution in binding of labeled BT cry toxin in the presence and absence of the toxin candidate.

In addition to simply screening candidates, the screen can be used to devise improved forms of toxins which are more specific or less specific to particular classes of insects as desired. The ability to determine binding affinity (K_a and K_d), dissociation and association rates, and cytotoxic effects of a candidate allows quick, accurate and reproducible screening techniques for a large number of toxins and other ligands under identical conditions which was not possible heretofore. Such information will facilitate the selection of the most effective toxins and ligands for any given receptor obtained from any desired host cell.

Competition assays may also employ antibodies that are specifically immunoreactive with the receptor. Such antibodies can be prepared in the conventional manner by administering the purified receptor to a vertebrate animal, monitoring antibody titers and recovering the antisera or the antibody-producing cells for immortalization, to obtain immortalized cells capable of secreting antibodies of the appropriate specificity. Techniques for obtaining immortalized B cells and for screening them for secretion of the desired antibody are now conventional in the art. The resulting monoclonal antibodies may be more effective than the polyclonal antisera as competition reagents; furthermore, the availability of the immortalized cell line secreting the desired antibody assures uniformity of production of the same reagent over time. The information and the structural characteristics of toxins and ligands tested will permit a rational approach to designing more efficient toxins and ligands. Additionally, such assays will lead to a better understanding of the function and the structure/function relationship of both toxin/ligand and BT-R_1 analogs. In turn, this will allow the development of highly effective toxins/ligands. Ligands include natural and modified toxins, antibodies (anti-receptor and antiidiotypic antibodies which mimic a portion of a toxin that binds to a receptor, and whatever small molecules bind the receptors.

I. Uses of Agents that Bind to a BT-R_1 Protein

As provided in the Background section, BT-R_1 is the target for the insecticidal activity of BT-toxins. Agents that bind a BT-R_1 protein can be used: 1) to kill BT-R_1 expressing cells, 2) to identify agents that block the interaction of a BT-toxin with BT-R_1 and 3) in methods for identifying cells that express BT-R_1.

The methods employed in using the BT-R_1 binding agents will be based primarily on the nature of the BT-R_1 binding agent and its intended use. For example, a BT-R_1 binding agent can be used to: deliver a conjugated toxin to a BT-R_1 expressing cell; modulate BT-R_1 activity; directly kill BT-R_1 expressing cells; or screen for and identify competitive binding agents. An agent that inhibits the activity of BT-R_1 can be used to directly inhibit the growth of BT-R_1 expressing cells. Further, identified cellular factors that bind to BT-R_1 can, themselves, be used in binding/competitive assays to identify agonist and antagonists of BT-R_1.

J. Methods for Identifying the Presence of a BT-R_1 protein or gene

The present invention further provides methods for identifying cells, tissues or organisms expressing a BT-R_1 protein or a *BT-R1* gene. Such methods can be used to diagnose the presence of cells or an organism that expresses a BT-R_1 protein *in vivo* or *in vitro*. The methods of the present invention are particularly useful in the determining the presence of cells that are a target for BT-toxin activity or for identifying susceptibility of an organism to a BT-toxin or BT-toxin-like agent. Specifically, the presence of a BT-R_1 protein can be identified by determining whether a BT-R_1 protein, or nucleic acid encoding a BT-R_1 protein, is expressed in a cell, tissue or organism.

A variety of immunological and molecular genetic techniques can be used to determine if a BT-R_1 protein is expressed/produced in a particular cell or sample. In general, an extract containing nucleic acid molecules or an extract containing proteins is prepared. The extract is then assayed to determine whether a BT-R_1 protein, or a BT-R_1 encoding nucleic acid molecule, is produced in the cell.

For example, to perform a diagnostic test based on nucleic acid molecules, a suitable nucleic acid sample is obtained and prepared using conventional techniques. DNA can be prepared, for example, simply by boiling a sample in SDS. The extracted nucleic acid can then be subjected to amplification, for example by using the polymerase chain reaction (PCR) according to standard procedures, such as a RT-PCR method, to selectively amplify a BT-R_1 encoding nucleic acid molecule or fragment thereof. The size or presence of a specific amplified fragment (typically following restriction endonuclease digestion) is then determined using gel electrophoresis or the nucleotide sequence of the fragment is determined (for example, see Weber and May *Am J Hum Genet* (1989) 44:388-339; Davies, J. *et al. Nature* (1994) 371:130-136)). The resulting size of the fragment or sequence is then compared to the known BT-R_1 proteins encoding sequences, for example via hybridization probe. Using this method, the presence of a BT-R_1 protein can be identified.

To perform a diagnostic test based on proteins, a suitable protein sample is obtained and prepared using conventional techniques. Protein samples can be prepared, for example, simply by mixing a sample with SDS followed by salt precipitation of a protein fraction. The extracted protein can then be analyzed to determine the presence of a BT-R_1 protein using known methods. For example, the presence of specific sized or charged variants of a protein can be identified using mobility in an electric filed. Alternatively, antibodies can be used for detection purposes. A skilled artisan can readily adapt known protein analytical methods to determine if a sample contains a BT-R_1 protein.

Alternatively, BT-R_1 protein or gene expression can also be used in methods to identify agents that decrease the level of expression of a *BT-R1* gene. For example, cells or tissues expressing a BT-R_1 protein can be contacted with a test agent to determine the effects of the agent on BT-R_1 protein/gene expression. Agents that activate BT-R_1 protein/gene expression can be used as an agonist of BT-R_1 activity whereas agents that decrease BT-R_1 protein/gene expression can be used as an antagonist of BT-R_1 activity.

K. Methods to Sensitize Cells

The present invention further provides methods of sensitizing cells such that they become susceptible to killing with a BT-toxin, or a BT-toxin analog. Specifically, host cells transformed to express BT-R_1 receptor, or a homolog of the BT-R_1 receptor, become sensitive to the mode of action of BT-toxins. The binding of a BT-toxin to a BT-R_1 receptor expressed on the surface of the transformed cells results in induction of a cellular death and apoptosis of the cell expressing the BT-R_1 receptor. Accordingly, the BT-R_1 receptor is an appropriate candidate for use in transforming cells in which it is desirable to induce cell death.

There are numerous situations in which it is desirable to introduce the selected gene into a selected population of cells, thus bringing about cell death. One such example is in the therapeutic treatment of cancer cells. In using specifically targeted vectors for delivery of BT-R_1-encoding DNA molecules into a tumor cell, tumor cells within a patient can be engineered to express a BT-R_1 protein. Such cells then become susceptible to death upon treatment with a BT-toxin. Since BT-toxin is not normally toxic to mammalian cells, this method is particularly applicable to inducing cell death in particular cells in a mammalian host. Other situations where it may be desirable to stimulate cell death in particular cells or cell lines are in the treatment of autoimmune disorders and in the treatment of cells harboring pathogens, such as malaria or HIV agents.

The choice of the actual steps employed to introduce a BT-R_1-encoding DNA molecule into a cell to render the cells susceptible to treatment with BT-toxin is based primarily on the cell type that is to be altered, the conditions under which the cell type will be altered, and the overall use envisioned. A skilled artisan can readily adapt art-known methods for use with the BT-R_1-encoding DNA molecule of the present invention.

L. Animal Models and Gene Therapy

The *BT-R1* gene and the BT-R_1 protein can also serve as a target for generating transgenic organisms in which the pattern of BT-R_1 expression has been altered. For

example, in one application, BT-R_1 deficient insects or insect cells can be generated using standard knock-out procedures to inactivate a *BT-R1* gene, or, if such animals are non-viable, inducible *BT-R1* antisense molecules can be used to regulate *BT-R1* activity/expression. Alternatively, cells or an organism can be altered so as to contain a *Manduca sexta* BT-R_1 encoding nucleic acid molecule or an antisense-BT-R_1 expression unit that directs the expression of a BT-R_1 protein or an antisense molecule in a tissue specific fashion. In such uses, an organism or cells, for example insects or insect cells, is generated in which the expression of a *BT-R1* gene is altered by inactivation or activation and/or replaced by a *Manduca sexta BT-R1* gene. This can be accomplished using a variety of art-known procedures such as targeted recombination. Once generated, the *BT-R1* expression altered cells or organisms can be used to 1) identify biological and pathological processes mediated by the BT-R_1 protein, 2) identify proteins and other genes that interact with the BT-R_1 protein, 3) identify agents that can be exogenously supplied to overcome a BT-R_1 protein deficiency and 4) serve as an appropriate screen for identifying mutations within the *BT-R1* gene that increases or decreases activity.

For example, it is possible to generate transgenic insects, such as members of the dipteran order, expressing the *Manduca sexta* minigene encoding BT-R_1 in a tissue specific-fashion and test the effect of over-expression of the protein in tissues and cells that normally do not contain the BT-R_1 protein.

M. Use of Expression Control Elements of the BT-R_1 Gene

The present invention further provides the expression control sequences found 5' of the of the newly identified *BT-R1* gene in a form that can be used in generating expression vectors. Specifically, the *BT-R1* expression control elements, such as the BT-R_1 promoter, that can readily be identified as being 5' from the ATG start codon in the *BT-R1* gene, can be used to direct the expression of an operably linked protein encoding DNA sequence. Since BT-R_1 expression is mostly tissue-specific, the expression control elements are particularly useful in directing the expression of an introduced transgene in a tissue specific fashion. A skilled artisan can readily use the

BT-R_1 gene promoter and other regulatory elements to generate expression vectors using methods known in the art.

Without further description, it is believed that one of ordinary skill in the art can, using the preceding description and the following illustrative examples, make and utilize the compounds of the present invention and practice the claimed methods. The following working examples therefore, specifically point out preferred embodiments of the present invention, and are not to be construed as limiting in any way the remainder of the disclosure.

Example 1

Purification and Sequence Determination of BT-R_1 Protein

Midguts of *M. sexta* were extracted and the BT-R_1 protein purified according to the method of Vadlamudi, R.K. *et al. J Biol Chem* (1993) 268:1233, referenced above and incorporated herein by reference. The electroeluted band was confirmed to contain BT-R_1 protein by binding to ^{125}I-*cryIAb* toxin. In gel electrophoresis, the protein bound to toxin had an apparent weight of approximately 210 kD under reducing and nonreducing conditions.

The purified electroeluted BT-R_1 was subjected to cyanogen bromide digestion and the cyanogen bromide fragments separated on a 17% high-resolution tricine SDS-polyacrylamide gel as described by Schagger, H. *et al. Anal Biochem* (1987) 166:368. The separated fragments were transferred to Problott membranes (Applied Biosystems) and five bands were extracted and subjected to microsequencing using standard instrumentation. The amino acid sequences obtained were:

1. (Met)-Leu-Asp-Tyr-Glu-Val-Pro-Glu-Phe-Gln-Ser-Ile-Thr-Ile-Arg-Val-Val-Ala-Thr-Asp-Asn-Asn-Asp-Thr-Arg-His-Val-Gly-Val-Ala;
2. (Met)-X-Glu-Thr-Tyr-Glu-Leu-Ile-Ile-His-Pro-Phe-Asn-Tyr-Tyr-Ala;
3. (Met)-X-X-X-His-Gln-Leu-Pro-Leu-Ala-Gln-Asp-Ile-Lys-Asn-His;
4. (Met)-Phe/Pro-Asn/Ile-Val-Arg/Tyr-Val-Asp-Ile/Gly;
5. (Met)-Asn-Phe-Phe/His-Ser-Val-Asn-Arg/Asp-Glu.

Example 2

Recovery of cDNA

An *M. sexta* cDNA library was constructed from midgut tissue in λgt10 using the Superscript Choice System according to the manufacturer's instructions (Life Technologies, Inc.). Degenerate oligonucleotide probes were constructed based on the peptide sequences determined in Example 1 using the methods and approach described in Zhang, S. *et al. Gene* (1991) 105:61. Synthetic oligonucleotides corresponding to peptides 1-3 of Example 1 were labeled with $\alpha^{32}P$ using polynucleotide kinase and used as probes as described in the standard cloning manual of Maniatis, T. *et al.* Molecular Cloning: A Laboratory Manual (Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, 2nd ed. 1989). A clone hybridizing to all three probes identified from 40 positive clones as hybridizing to all three of the probes was plaque-purified from a screen of 4 X 10^5 recombinants and subcloned into pBluescript (Stratagene). It contained an insert of 5571 bp.

Double-stranded cDNA in pBluescript was sequenced in both directions by the dideoxy termination method with Sequanase (USB) according to the manufacturer's instructions. The sequencing showed an open reading frame of 4584 base pairs or 1528 amino acids along with a polyadenylation signal at position 5561. The sequence obtained and the deduced amino acid sequence is shown in Figure 1.

Thus, the deduced protein has a molecular mass of 172 kD and a pI of approximately 4.5. The amino acid sequences of the cyanogen bromide fragments of native receptor match perfectly within the deduced amino acid sequence. The open reading frame begins with an ATG that is flanked by the consensus translation initiation sequence GAGATGG for eucaryotic mRNAs as described by Kozak, M. *Nucleic Acids Res* (1987) 15:8125.

As shown in Figure 1, the deduced amino acid sequence includes a putative signal, shown underlined, preceding the mature N-terminus Asn-Glu-Arg-etc. Eleven repeats (cad1-cad11) are shown in the extracellular region upstream of the membrane domain, shown with the heavy underline, at positions 1406-1427. The end of the 11th

repeat is shown with an arrowhead. The positions of the five CNBR fragments are also shown under the complete sequence.

Figure 2 compares the BT-R_1 sequence obtained herein with other members of the cadherin family. Like known cadherins, the external domain of BT-R_1 is highly repetitive and contains 11 repeats (cad1-cad11; see Figure 2 A). The other cadherins compared in Figure 2 B are mouse P cadherin (mP EC1); *Drosophila fat* EC18 (fat EC18) and protocadherin (PC42 EC2), and *Manduca sexta* intestinal transporter (HPT-1-EC-1). The eleven repeats of the cadherin motif in BT-R_1 (cad1-cad11) are individually aligned with a single motif sequence from each of the other members of the cadherin family. Conserved residues are boxed. The greatest similarity of BT-R_1 to the cadherins is with the extracellular repeats of the cadherin motif of mouse P-cadherin, Drosophila *fat* tumor suppressor and the protocadherins, although homologies are not high (20-40 homology and 30-60 percent similarity). The conserved repeats of BT-R_1 included AXDXD, DXE, DXNDXXP, one glutamic acid residue and two glycine residues (Figure 2 B). Motifs A/VXDXD, DXNDN are the consensus sequences for calcium binding and two such regions are present in a typical cadherin repeat. In all repeats of BT-R_1, the sequence DXNDN is preceded by 8 to 14 hydrophobic amino acids. Similar hydrophobic sequences also have been observed in the cadherins. The length of the hydrophobic stretches suggests that these areas are not transmembrane regions buy that the represent J-sheet structures commonly present in cadherin-like repeats. BT-R_1 contains a putative cytoplasmic domain of 101 amino acids, smaller than vertebrate cadherin cytoplasmic domains (160 amino acids), and shows no homology to any of the cadherin cytoplasmic domains or to cytoplasmic domains of other proteins to which it has been compared in a current sequence data base.

To confirm that the sequenced clone encoded full-length BT-R_1 protein, total mRNA was prepared from midguts of *M. sexta* subjected to Northern blot by hybridization with the antisense 4.8 kb SacI fragment of the BT-R_1 cDNA clone. The Northern blot analysis was conducted by hybridizing to the antisense probe at 42°C and 50% formamide, 5 X Denhardt's Reagent, 5 X SSCP and 50 µg/ml salmon sperm

DNA. The filter was then washed two times with 1 X SSC + 0.1% SDS and two times with 0.15 X SSC + 0.1% SDS at 42°C. Each wash was roughly 20 minutes. The filter was then exposed to X-ray film for 24 hours. The 4.8 kb probe hybridized to a single 5.6 kb band.

The BT-R_1 clone was translated using rabbit reticulolysate and the resulting translated products were immunoprecipitated with antisera raised against native protein encoded by BT-R_1. For the *in vitro* translation, pBluescript plasmid containing BT-R_1 cDNA was linearized and transcribed with T_3 polymerase (Pharmacia). The translation was conducted according to manufacturer's instructions with nuclease-treated rabbit reticulolysate (Life Technologies, Inc.). After one hour of incubation at 30°C, the reaction mixture was combined with an equal volume of SDS buffer or lysed with 50 mM Tris buffer containing 1% NP40 and 250 mM NaCl (pH 8.0) for immunoprecipitation. Preimmune serum was used as a control. Translation and immunoprecipitation products were electrophoresed on a 7.5% SDS-polyacrylamide gel fixed, treated with Enhance (Dupont NEN), dried and exposed to X-ray film for 12 hours.

Two protein bands of approximately 172 kD and 150 kD as determined by SDS-PAGE were obtained; it is postulated that the 150 kD translation product was due to initiation of translation from an internal methionine at amino acid 242. This is consistent with the observations of Kozak, M. *Mol Cell Biol* (1989) 9:5073.

Thus, both results confirm that a full-length clone was obtained.

Example 3

Recombinant Production and Characteristics of the BT-R_1 Protein

The BT-R_1 cDNA clone was subcloned into the mammalian expression vector pcDNA3 (Invitrogen) and the construct transfected into COS-7 cells. Membranes isolated from the COS-7 transfectants were solubilized, electrophoresed and ligand blotted with ^{125}I-*CryIAb* toxin. The cells were harvested 60 hours after transfection, washed with phosphate-buffered saline and lysed by freezing in liquid nitrogen. Cell membranes were prepared by differential centrifugation as described by Elshourbagy,

N.A. *et al. J Biol Chem* (1993) 266:3873. Control cells were COS-7 cells transfected with pcDNA3.

The cell membranes (10 µg) were separated on 7.5% SDS-PAGE blotted to a nylon membrane and blocked with Tris-buffered saline containing 5% nonfat dry milk powder, 5% glycerol and 1% Tween-20. The nylon membrane was then incubated with ^{125}I-*CryIAb* toxin (2 X 10^5 cpm/ml) for two hours with blocking buffer, dried and exposed to X-ray film at -70°C. The labeled toxin bound to a 210 ± 5 kD protein; the 210 kD band was observed only in lanes containing membranes prepared from either *M. sexta* or COS-7 cells transfected with the BT-R_1 cDNA construct containing 4810 bp of cDNA comprising the open reading frame.

The discrepancy between the 210 kD protein expressed and the calculated 172 kD molecular weight is due to glycosylation of the protein; *in vitro* translation of the cDNA clone, as described above, which does not result in glycosylation, does produce the 172 kD protein. To verify this, the COS-7 produced protein was subjected to digestion with N-glycosidase-F by first denaturing the purified protein by boiling in 1% SDS for 5 minutes followed by addition of NP-40 to a final concentration of 1% in the presence of 0.1% SDS, and then incubating the denatured protein in sodium phosphate buffer, pH 8.5 at 37°C with N-glycosidase-F for 10 hours. Controls were incubated under the same conditions without enzyme. Digestion products were separated on a 7.5% SDS-PAGE and stained with Coomassie brilliant blue. This glycosidase treatment reduced the molecular weight of BT-R_1 protein from 210 to 190 kD; this indicates N-glycosylation at some of the 16 consensus N-glycosylation sites in the protein. Treatment of BT-R_1 with O-glycosidase and neuraminidase did not alter the mobility of the protein.

In addition, embryonic 293 cells were transfected with the BT-R_1 cDNA clone in pcDNA3 and incubated with the labeled toxin (0.32 nM) in the presence of increasing concentrations (0 to 10^{-6} M) of unlabeled toxin. Nonspecific binding was measured as bound radioactivity in the presence of 1 TM unlabeled toxin. A value for the dissociation constant (K_d) of 1015 pM was determined by Scatchard analysis; this

is approximately the same value that was obtained for the natural receptor as described by Vadlamudi, R.K. *et al. J Biol Chem* (1993) (*supra*).

Example 4

Physiological Effect of BT Toxin on Modified Embryonic 293 Cells

Both unmodified embryonic 293 cells, and 293 cells which have been modified to produce the BT-R_1 receptor as described in Example 3, when cultured *in vitro* form adherent star-shaped clusters. When BT toxin (200 nM) is added to serum-free medium, the clusters round up and release from the plastic surfaces of the culture dish. This effect is also observed under known conditions of cytotoxicity for 293 cells. The foregoing effect is observed only when the cells are cultured in serum-free medium since the toxin binds to serum and would thus be ineffective under conditions where serum is present.

However, in the presence of anti-receptor antisera, this effect of BT toxin is blocked. Also, when serum is added back to a culture of modified E293 cells which has been treated in serum-free conditions with the toxin, the cells revert to their normal star-shaped adherent cluster shapes. This indicates that the effect of the toxin is reversible.

Example 5

Identification Of A Fragment Of BT-R_1 That Binds To A BT Toxin

To understand some of the properties of BT-R_1, research has been undertaken to define the location of the BT-R_1/Cry1Ab protein-protein interaction. The full-length wild-type amino acid sequence of BT-R_1 is provided in Fig. 1 with a block diagram of a possible cadherin-like structure for BT-R_1 shown in Fig 3. In both figures, restriction digest sites from the cDNA are provided relative to the positions at which they would disrupt the amino acid coding sequence.

A small fragment lying between the BamHI and SacI restriction sites of wild-type BT-R_1 was cloned into the vector pCITE (Novagen). This vector contains transcription/translation sequences designed for use in a rabbit reticulocyte lysate

(RRL) system. The clone has been analyzed by restriction mapping and mRNA expression (Fig. 4). Lane UP shows the uncut plasmid and lanes NP and XP show restriction digests using NsiI and XhoI, respectively. NsiI is used because it has only one restriction site lying within the Bam-Sac fragment and does not cut anywhere within the pCITE vector. The BSP lane shows the restriction digest of the clone using BamHI and SacI. The digest releases the cloned fragment which separates at about 700 base pairs. The RT1 and RT2 lanes show mRNA transcription from the clone after linearization with XhoI. The mRNA separates at the expected 1350 base pairs.

Protein for analysis has been prepared from this clone in two ways. First, an RRL translation kit was employed to produce protein from the mRNA transcription reaction described above. Second, the plasmid was added directly to an RRL based transcription and translation (TNT) coupled kit. Protein production was detected using ^{35}S-methionine as a tag (Fig. 5). The LCR lane shows production of luciferase protein from mRNA in an RRL kit and the LCT lane is luciferase protein from a plasmid containing the luciferase coding sequence translated in the TNT kit. Both are positive controls to demonstrate that the two translation kits are operational. The major bands for luciferase translation are observed at 66 kDa. The lanes labeled as RR_1 and RR2 show expression of the polypeptide sequence of the Bam-Sac fragment of BT-R_1 translated from mRNA in the RRL kit. The lanes TT1 and TT2 are translations from the pCITE plasmid containing the Bam-Sac fragment from the TNT kit. All four lanes possess a major band at 30 kDa which is the expected size of the Bam-Sac fragment with the addition of a coded antibody tag called S-tag. S-tag is part of the multicloning site of pCITE.

The clone was then tested for its ability to bind the insecticidal toxin Cry1Ab. Polypeptide translation of the Bam-Sac fragment of BT-R_1 was carried out in duplicate as described above. The only change is that the ^{35}S-methionine tag was left out of the reaction mixtures to produce non-radiolabeled proteins. The proteins were separated by SDS-PAGE, blotted to nitrocellulose and hybridized with ^{125}I-labeled Cry1Ab (Fig. 6). BBMV is wild-type BT-R_1 prepared from the midgut brush border

membrane vesicles (BBMV) of *M. sexta,* and, is used as a positive control. RBK and TBK are RRL and TNT control reactions prepared without mRNA or plasmid present to determine whether proteins endogenous to either kit bind Cry1Ab. R_1 and RR2 are translations from the RRL kit and TT1 and TT2 are from the TNT kit. A single 30-kDa band appears in each of these lanes. Two are marked by arrows. These bands demonstrate that the Bam-Sac fragment of BT-R_1 is capable of binding Cry1Ab insecticidal toxin.

To further understand the nature of this binding site, a set of truncation mutants of BT-R_1 was prepared through the use of restriction digests. The cDNA was digested at specific sites to remove increasingly larger portions of the C-terminus. The restriction enzymes used were NsiI, BamHI, NruI, ClaI, XhoI and StuI (Figs. 1 and 3). The procedure involved linearizing the plasmid at each one of these sites and transcribing up to the truncation. The shortened mRNAs then were translated in an RRL kit blotted to nitrocellulose and hybridized with ^{125}I-labeled Cry1Ab. Translation of the wild-type BT-R_1 from the cDNA showed binding to a 172-kDa protein band, the expected size of wild-type BT-R_1. It also shows smaller bands that bind Cry1Ab although the nature of these bands has not been determined. A blank made by preparing an RRL reaction mixture without any mRNA gaves several bands below 66 kDa that show some type of binding of Cry1Ab to the reticulocytes. The specificity of this binding has not been determined. The truncation mutants created by NsiI, BamHI, NruI, ClaI, XhoI and StuI restriction digests did not show any binding to Cry1Ab except in the region where the reticulocytes bind Cry1Ab. This data demonstrates that the removal of the last 100 amino acids from wild type BT-R_1 by NsiI restriction results in the loss of the ability of BT-R_1 to bind Cry1Ab. This localizes the toxin binding site on the BT-R_1 clone and provides a soluble fragment of the receptor that can be used in toxin and other binding studies.

A clone of a fragment of BT-R_1, called the Bam-Sac fragment, has been prepared. It was prepared using BamHI and SacI restriction digests (Fig. 1) and cloning of the resulting fragment into a vector called pCITE. The polypeptide sequence was translated and tested for binding to the insecticidal toxin Cry1Ab

(Figure 8). The Bam-Sac fragment binds to Cry1Ab, providing first insight into the location of the Cry1Ab binding site within the BT-R_1 sequence. It lies in the last 234 C-terminal amino acids. This evidence is further supported by a set of truncation mutants that has been prepared. Removal of the 100 most C-terminal amino acids from wild type BT-R_1 results in the loss of Cry1Ab binding. The C-terminal end of BT-R_1 is the location of the Cry1Ab binding site.

Example 6

Identification Of Homologue of BT-R_1 That Binds To A BT Toxin

Western blots of tissue extracts prepared from Pink bollworm and European corn borer were prepare and probed with labeled Cry1a (Figure 7). The results show that homologues of BT-R_1 are present in these two insects and can be readily isolated using the methods described herein.

Claims

1. A method to identify agents that bind to a BT-toxin receptor, said method comprising the steps of:

i) contacting an agent with a BT-toxin binding receptor selected from the group consisting of a) a cell that has been altered to contain a nucleic acid molecule that encodes the amino acid sequence of SEQ ID No:2, b) a cell that has been altered to contain a nucleic acid molecule that encodes a fragment of the amino acid sequence of SEQ ID No:2 that binds to a BT toxin, c) a cell that has been altered to contain a nucleic acid molecule encoding a BT-toxin receptor that hybridizes to the nucleic acid sequence of SEQ ID No:1 under high stringency, d) a cell that has been altered to contain a nucleic acid molecule that encodes a fragment of a BT-toxin receptor that hybridizes to the nucleic acid sequence of SEQ ID No:1 under high stringency and that binds to a BT toxin, e) an isolated protein with an amino acid sequence of SEQ ID No:2, f) an isolated fragment of a protein with an amino acid sequence of SEQ ID No:2, said fragment containing a BT-toxin binding domain, g) an isolated BT-toxin receptor that is encoded by a nucleic acid molecule that hybridizes to the nucleic acid sequence of SEQ ID No:1 under high stringency, and h) an isolated fragment of a BT-toxin receptor that is encoded by a nucleic acid molecule that hybridizes to the nucleic acid sequence of SEQ ID No:1 under high stringency, and

ii) determining whether said agent binds to said BT-toxin receptor.

2. The method of claim 1, wherein said method further comprises the step of determining whether said agent blocks the binding of a BT-toxin to said BT-toxin receptor.

3. The method of claim 1, wherein said cell that has been altered is a eukaryotic cell.

4. The method of claim 3, wherein eukaryotic cell is an insect cell.

5. A method to identify agents that block the binding of a BT-toxin to a BT-toxin receptor, said method comprising the steps of:

i) contacting an agent, in the presence and absence of a BT-toxin, with a BT-toxin binding receptor selected from the group consisting of a) a cell that has been altered to contain a nucleic acid molecule that encodes the amino acid sequence of SEQ ID No:2, b) a cell that has been altered to contain a nucleic acid molecule that encodes a fragment of the amino acid sequence of SEQ ID No:2 that binds to a BT toxin, c) a cell that has been altered to contain a nucleic acid molecule encoding a BT-toxin receptor that hybridizes to the nucleic acid sequence of SEQ ID No:1 under high stringency, d) a cell that has been altered to contain a nucleic acid molecule that encodes a fragment of a BT-toxin receptor that hybridizes to the nucleic acid sequence of SEQ ID No:1 under high stringency and that binds to a BT toxin, e) an isolated protein with an amino acid sequence of SEQ ID No:2, f) an isolated fragment of a protein with an amino acid sequence of SEQ ID No:2, said fragment containing a BT-toxin binding domain, g) an isolated BT-toxin receptor that is encoded by a nucleic acid molecule that hybridizes to the nucleic acid sequence of SEQ ID No:1 under high stringency, and h) an isolated fragment of a BT-toxin receptor that is encoded by a nucleic acid molecule that hybridizes to the nucleic acid sequence of SEQ ID No:1 under high stringency, and

ii) determining whether said agent blocks the binding of said BT-toxin to said BT-toxin receptor.

6. The method of claim 5, wherein said BT-toxin is a member of the BT-cry(1) family of toxins.

7. The method of claim 5, wherein said cell that has been altered is a eukaryotic cell.

8. The method of claim 7, wherein eukaryotic cell is an insect cell.

9. An isolated antibody, wherein said antibody binds to a protein selected from the group consisting of a) a BT-toxin receptor protein with an amino acid sequence of SEQ ID No:2, and b) a BT-toxin receptor protein that is encoded by a nucleic acid molecule that hybridizes to the nucleic acid sequence of SEQ ID No:1 under high stringency, or a fragment of said antibody, wherein said antibody fragment binds to said BT-toxin.

10. The antibody of claim 9, wherein said antibody binds to said BT-toxin receptor and blocks the binding of a BT-toxin to said receptor.

11. The antibody of claim 10, wherein said antibody binds to an epitope located within the 232 c-terminal amino acids of the BT-toxin receptor depicted in SEQ ID No:2.

12. An isolated BT-toxin receptor protein selected from the group consisting of a) a BT-toxin receptor protein with an amino acid sequence of SEQ ID No:2, b) a BT-toxin receptor protein that is encoded by a nucleic acid molecule that hybridizes to the nucleic acid sequence of SEQ ID No:1 under high stringency, c) a fragment of a BT-toxin receptor protein with an amino acid sequence of SEQ ID No:2, said fragment being able to bind to a BT-toxin, and d) a fragment of a BT-toxin receptor protein that is encoded by a nucleic acid molecule that hybridizes to the nucleic acid sequence of SEQ ID No:1 under high stringency, said fragment being able to bind to a BT-toxin.

13. A method to produce BT-toxin receptor protein, or a fragment thereof, said method comprising the steps of:

i) culturing a cell that has been altered to contain a nucleic acid molecule that encodes a BT-toxin receptor protein, of BT-toxin binding fragment thereof, wherein said cell has been altered to contain a nucleic acid molecule selected from the

group consisting of a) a nucleic acid molecule that encodes the amino acid sequence of SEQ ID No:2, b) a nucleic acid molecule that encodes a fragment of the amino acid sequence of SEQ ID No:2 that binds to a BT toxin, c) a nucleic acid molecule encoding a BT-toxin receptor that hybridizes to the nucleic acid sequence of SEQ ID No:1 under high stringency, and d) a nucleic acid molecule that encodes a fragment of a BT-toxin receptor that hybridizes to the nucleic acid sequence of SEQ ID No:1 under high stringency and that binds to a BT toxin, under condition in which said nucleic acid molecule is expressed and

ii) isolating said BT-toxin receptor protein or fragment.

14. The method of claim 13, wherein said cell that has been altered is a eukaryotic cell.

15. The method of claim 14, wherein eukaryotic cell is an insect cell.

Sequence Range: 1 to 5578

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          10         20         30         40         50         60         70         80         90        100        110        120
          .          .          .          .          .          .          .          .          .          .          .          .
GACCAATCGGAGTGTGGTGAATTTTTGGAAAATATTTTGTGCGGTTCCTTTAGTTGTGTAATATAGTACTTTAGTTACAAATTTGGAATAATTTGGCAGCAAAACCATCTGCAGCAACAA

          130        140        150        160        170        180        190        200        210        220
          .          .          .          .          .          .          .          .          .          .
AATCATCTGCAGCTGCGAAATCATCTGCAGCAGCAAAAGCATCTTCAGGAGCGAGAAAAGCCCCAAATAATGTGAG ATG GCA GTT GAC GTC CGA ATC GCT GCC TTC CTG
                                                                                 Met Ala Val Asp Val Arg Ile Ala Ala Phe Leu

230        240        250        260        270        280        290        300        310
.          .          .          .          .          .          .          .          .
CTG GTG TTT ATA GCG CCT GCA GTT TTA GCT CAA GAG AGA TGT GGG TAT ATG ACC GCC ATC CCA AGG CTA CCA CGA CCG GAT AAT TTG CCA
Leu Val Phe Ile Ala Pro Ala Val Leu Ala Gln Glu Arg Cys Gly Tyr Met Thr Ala Ile Pro Arg Leu Pro Arg Pro Asp Asn Leu Pro

320        330        340        350        360        370        380        390        400
.          .          .          .          .          .          .          .          .
GTA CTA AAT TTT GAA GGC CAG ACA TGG AGT CAG AGG CCC CTG CTC CCC GCC CCG GAG CGG GAT GAC CTG TGC ATG GAC GCC TAC CAC GTG
Val Leu Asn Phe Glu Gly Gln Thr Trp Ser Gln Arg Pro Leu Leu Pro Ala Pro Glu Arg Asp Asp Leu Cys Met Asp Ala Tyr His Val

410        420        430        440        450        460        470        480        490
.          .          .          .          .          .          .          .          .
ATA ACA GCC AAC CTC GGC ACG CAG GTC ATC TAC ATG GAT GAA GAG ATA GAA GAC GAA ATC ACC ATC GCC ATA CTT AAT TAT AAC GGA CCA
Ile Thr Ala Asn Leu Gly Thr Gln Val Ile Tyr Met Asp Glu Glu Ile Glu Asp Glu Ile Thr Ile Ala Ile Leu Asn Tyr Asn Gly Pro

500        510        520        530        540        550        560        570        580
.          .          .          .          .          .          .          .          .
TCA ACT CCG TTC ATT GAA CTG CCA TTT TTA TCC GGT TCG TAC AAT CTG CTG ATG CCG GTC ATC AGG AGA GTT GAC AAC GGG AGT GCA TCT
Ser Thr Pro Phe Ile Glu Leu Pro Phe Leu Ser Gly Ser Tyr Asn Leu Leu Met Pro Val Ile Arg Arg Val Asp Asn Gly Ser Ala Ser

590        600        610        620        630        640        650        660        670
.          .          .          .          .          .          .          .          .
CAT CAT CAC GCA AGA CAG CAT TAC GAG TTG CCC GGC ATG CAG CAG TAC ATG TTC AAT GTG CGC GTG GAC GGC CAG TCG CTG GTG GCA GGC
His His His Ala Arg Gln His Tyr Glu Leu Pro Gly Met Gln Gln Tyr Met Phe Asn Val Arg Val Asp Gly Gln Ser Leu Val Ala Gly

680        690        700        710        720        730        740        750        760
.          .          .          .          .          .          .          .          .
GTG TCT CTC GCT ATC GTC AAC ATA GAT GAC AAC GCG CCC ATC ATA CAA AAC TTC GAG CCT TGC CGG GTT CCT GAA CTG GGC GAG CCA GGG
Val Ser Leu Ala Ile Val Asn Ile Asp Asp Asn Ala Pro Ile Ile Gln Asn Phe Glu Pro Cys Arg Val Pro Glu Leu Gly Glu Pro Gly

770        780        790        800        810        820        830        840        850
.          .          .          .          .          .          .          .          .
TTG ACA GAA TGC ACA TAC CAA GTA TCG GAC GCG GAC GGA CGG ATC AGC ACA GAG TTC ATG ACG TTC AGG ATC GAC AGC GTT CGT GGC GAC
Leu Thr Glu Cys Thr Tyr Gln Val Ser Asp Ala Asp Gly Arg Ile Ser Thr Glu Phe Met Thr Phe Arg Ile Asp Ser Val Arg Gly Asp

860        870        880        890        900        910        920        930        940
.          .          .          .          .          .          .          .          .
GAG GAG ACC TTC TAC ATC GAA CGG ACG AAT ATC CCC AAC CAA TGG ATG TGG CTA AAT ATG ACC ATA GGC GTT AAT ACC TCG CTC AAC TTC
Glu Glu Thr Phe Tyr Ile Glu Arg Thr Asn Ile Pro Asn Gln Trp Met Trp Leu Asn Met Thr Ile Gly Val Asn Thr Ser Leu Asn Phe

950        960        970        980        990       1000       1010       1020       1030
.          .          .          .          .          .          .          .          .
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Fig 1.

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   GTC ACC AGT CCG CTG CAT ATA TTC AGC GTG ACA GCC CTG GAC TCG CTC CCG AAC ACC CAC ACG GTG ACT ATG ATG GTC CAA GTG GCG AAT
   Val Thr Ser Pro Leu His Ile Phe Ser Val Thr Ala Leu Asp Ser Leu Pro Asn Thr His Thr Val Thr Met Met Val Gln Val Ala Asn-

1040        1050        1060        1070        1080        1090        1100        1110        1120
   .           .           .           .           .           .           .           .           .
   GTG AAC AGC CGT CCG CCG CGC TGG CTG GAG ATC TTC GCT GTC CAA CAG TTT GAA GAG AAA TCT TAC CAA AAC TTC ACA GTG AGG GCG ATC
   Val Asn Ser Arg Pro Pro Arg Trp Leu Glu Ile Phe Ala Val Gln Gln Phe Glu Glu Lys Ser Tyr Gln Asn Phe Thr Val Arg Ala Ile-

1130        1140        1150        1160        1170        1180        1190        1200        1210
   .           .           .           .           .           .           .           .           .
   GAC GGA GAC ACT GAG ATC AAT ATG CCT ATC AAC TAC AGG CTG ATC ACA AAT GAG GAA GAC ACA TTC TTC AGC ATT GAG GCC CTG CCT GGT
   Asp Gly Asp Thr Glu Ile Asn Met Pro Ile Asn Tyr Arg Leu Ile Thr Asn Glu Glu Asp Thr Phe Phe Ser Ile Glu Ala Leu Pro Gly-

1220        1230        1240        1250        1260        1270        1280        1290        1300
   .           .           .           .           .           .           .           .           .
   GGA AAA AGC GGG GCT GTA TTC CTC GTG TCG CCA ATT GAC CGC GAC ACA CTG CAA CGA GAG GTG TTT CCA CTT ACG ATC GTC GCT TAC AAA
   Gly Lys Ser Gly Ala Val Phe Leu Val Ser Pro Ile Asp Arg Asp Thr Leu Gln Arg Glu Val Phe Pro Leu Thr Ile Val Ala Tyr Lys-

1310        1320        1330        1340        1350        1360        1370        1380        1390
   .           .           .           .           .           .           .           .           .
   TAT GAT GAG GAG GCC TTC TCC ACA TCA ACA AAC GTG GTC ATC ATT GTG ACA GAC ATC AAC GAC CAA AGA CCT GAA CCT ATA CAC AAG GAA
   Tyr Asp Glu Glu Ala Phe Ser Thr Ser Thr Asn Val Val Ile Ile Val Thr Asp Ile Asn Asp Gln Arg Pro Glu Pro Ile His Lys Glu-

1400        1410        1420        1430        1440        1450        1460        1470        1480
   .           .           .           .           .           .           .           .           .
   TAT CGA CTG GCA ATC ATG GAG GAG ACG CCC CTG ACC CTC AAC TTC GAT AAA GAA TTC GGA TTT CAT GAT AAG GAT TTA GGT CAA AAC GCT
   Tyr Arg Leu Ala Ile Met Glu Glu Thr Pro Leu Thr Leu Asn Phe Asp Lys Glu Phe Gly Phe His Asp Lys Asp Leu Gly Gln Asn Ala-

1490        1500        1510        1520        1530        1540        1550        1560        1570
   .           .           .           .           .           .           .           .           .
   CAG TAC ACG GTG CGT CTA GAG AGC GTG GAC CCT CCA GGC GCT GCT GAG GCA TTC TAC ATA GCG CCT GAA GTC GGC TAC CAG CGA CAG ACC
   Gln Tyr Thr Val Arg Leu Glu Ser Val Asp Pro Pro Gly Ala Ala Glu Ala Phe Tyr Ile Ala Pro Glu Val Gly Tyr Gln Arg Gln Thr-

1580        1590        1600        1610        1620        1630        1640        1650        1660
   .           .           .           .           .           .           .           .           .
   TTC ATC ATG GGC ACC CTC AAT CAC TCC ATG CTG GAT TAC GAA GTG CCA GAG TTT CAG AGT ATT ACG ATT CGG GTG GTA GCG ACC GAC AAC
   Phe Ile Met Gly Thr Leu Asn His Ser Met Leu Asp Tyr Glu Val Pro Glu Phe Gln Ser Ile Thr Ile Arg Val Val Ala Thr Asp Asn-

1670        1680        1690        1700        1710        1720        1730        1740        1750
   .           .           .           .           .           .           .           .           .
   AAC GAC ACG AGG CAC GTG GGC GTC GCG TTG GTT CAC ATT GAC CTC ATC AAT TGG AAC GAT GAG CAG CCG ATC TTC GAA CAC GCC GTG CAG
   Asn Asp Thr Arg His Val Gly Val Ala Leu Val His Ile Asp Leu Ile Asn Trp Asn Asp Glu Gln Pro Ile Phe Glu His Ala Val Gln-

1760        1770        1780        1790        1800        1810        1820        1830        1840
   .           .           .           .           .           .           .           .           .
   ACC GTC ACC TTC GAC GAG ACT GAA GGC GAG GGG TTC TTC GTC GCC AAG GCG GTT GCA CAC GAC AGA GAC ATC GGG GAT GTC GTC GAG CAT
   Thr Val Thr Phe Asp Glu Thr Glu Gly Glu Gly Phe Phe Val Ala Lys Ala Val Ala His Asp Arg Asp Ile Gly Asp Val Val Glu His-

1850        1860        1870        1880        1890        1900        1910        1920        1930
   .           .           .           .           .           .           .           .           .
   ACT TTA TTG GGT AAC GCT GTT AAC TTC CTG ACC ATC GAC AAA CTC ACC GGC GAC ATC CGC GTC TCA GCT AAC GAC TCC TTC AAC TAC CAT
   Thr Leu Leu Gly Asn Ala Val Asn Phe Leu Thr Ile Asp Lys Leu Thr Gly Asp Ile Arg Val Ser Ala Asn Asp Ser Phe Asn Tyr His-

1940        1950        1960        1970        1980        1990        2000        2010        2020
```

FIG 16 X1

CGA GAA AGT GAA TTA TTT GTG CAG GTG CGA GCT ACA GAC ACG CTG GGC GAA CCC TTC CAC ACG GCG ACG TCA CAG CTG GTC ATA CGA CTA
Arg Glu Ser Glu Leu Phe Val Gln Val Arg Ala Thr Asp Thr Leu Gly Glu Pro Phe His Thr Ala Thr Ser Gln Leu Val Ile Arg Leu

2030 2040 2050 2060 2070 2080 2090 2100 2110

AAT GAC ATC AAC AAC ACG CCA CCC ACC TTA CGG CTG CCT CGA GGC AGT CCC CAA GTG GAG GAG AAC GTG CCT GAT GGC CAC GTC ATC ACC
Asn Asp Ile Asn Asn Thr Pro Pro Thr Leu Arg Leu Pro Arg Gly Ser Pro Gln Val Glu Glu Asn Val Pro Asp **Gly** His Val Ile Thr

2120 2130 2140 2150 2160 2170 2180 2190 2200

CAG GAG TTA CGC GCC ACC GAC CCC GAC ACC ACG GCC GAT CTG CGC TTC GAG ATA AAC TGG GAC ACC TCT TTC GCC ACC AAG CAA GGC CGC
Gln Glu Leu Arg Ala Thr Asp Pro Asp Thr Thr Ala Asp Leu Arg Phe Glu Ile Asn Trp Asp Thr Ser Phe Ala Thr Lys Gln Gly Arg

2210 2220 2230 2240 2250 2260 2270 2280 2290

CAG GCT AAC CCC GAC GAG TTT AGG AAT TGC GTG GAA ATC GAG ACC ATC TTC CCC GAG ATT AAC AAC CGG GGA CTG GCT ATC GGC CGC GTT
Gln Ala Asn Pro Asp Glu Phe Arg Asn Cys Val Glu Ile Glu Thr Ile Phe Pro Glu Ile Asn Asn Arg Gly Leu Ala Ile Gly Arg Val

2300 2310 2320 2330 2340 2350 2360 2370 2380

GTA GCG CGC GAA ATC AGA CAC AAC GTG ACC ATA GAC TAC GAG GAG TTT GAG GTC CTC TCC CTC ACA GTG AGG GTG CGT GAC CTT AAC ACC
Val Ala Arg Glu Ile Arg His Asn Val Thr Ile Asp Tyr Glu Glu Phe Glu Val Leu Ser Leu Thr Val Arg Val Arg Asp Leu Asn Thr

2390 2400 2410 2420 2430 2440 2450 2460 2470

GTC TAC GGA GAC GAC TAC GAC GAA TCG ATG CTC ACA ATA ACT ATA ATC GAT ATG AAC GAC AAC GCG CCG GTG TGG GTG GAG GGA ACT CTG
Val Tyr Gly Asp Asp Tyr Asp Glu Ser Met Leu Thr Ile Thr Ile Ile Asp Met Asn Asp Asn Ala Pro Val Trp Val Glu Gly Thr Leu

2480 2490 2500 2510 2520 2530 2540 2550 2560

GAG CAG AAC TTC CGA GTC CGC GAG ATG TCG GCG GGC GGG CTC GTG GTG GGC TCC GTG CGC GCG GAC GAC ATC GAC GGA CCG CTC TAC AAC
Glu Gln Asn Phe Arg Val Arg Glu Met Ser Ala Gly Gly Leu Val Val Gly Ser Val Arg Ala Asp Asp Ile Asp Gly Pro Leu Tyr Asn

2570 2580 2590 2600 2610 2620 2630 2640 2650

CAA GTG CGA TAC ACC ATT TTC CCT CGT GAA GAC ACA GAT AAG GAC CTG ATA ATG ATC GAC TTC CTC ACG GGT CAA ATT TCC GTG AAC ACA
Gln Val Arg Tyr Thr Ile Phe Pro Arg Glu Asp Thr Asp Lys Asp Leu Ile Met Ile <u>Asp Phe Leu Thr</u> Gly <u>Gln Ile Ser Val Asn Thr</u>

2660 2670 2680 2690 2700 2710 2720 2730 2740

AGC GGC GCG ATC GAC GCG GAT ACT CCT CCA CGC TTC CAC CTC TAC TAT ACA GTG GTC GCT AGT GAC CGA TGC TCG ACA GAA GAT CCT GCA
<u>Ser Gly Ala</u> Ile Asp Ala <u>Asp</u> Thr Pro Pro Arg Phe His Leu Tyr Tyr Thr Val Val Ala Ser Asp Arg Cys Ser Thr Glu Asp Pro Ala

2750 2760 2770 2780 2790 2800 2810 2820 2830

GAT TGC CCC CCT GAC CCG ACT TAT TGG GAA ACC GAA GGA AAT ATC ACA ATC CAC ATC ACC GAC ACG AAC AAC AAG GTC CCG CAG GCG GAA
Asp Cys Pro Pro Asp Pro Thr Tyr Trp Glu Thr Glu Gly Asn Ile Thr Ile His Ile Thr Asp Thr Asn Asn Lys Val Pro Gln Ala Glu

2840 2850 2860 2870 2880 2890 2900 2910 2920

ACG ACT AAG TTC GAT ACC GTC GTG TAT ATT TAC GAG AAC GCA ACC CAC TTA GAC GAG GTG GTC ACT CTG ATA GCC AGT GAT CTT GAC AGA
Thr Thr Lys Phe Asp Thr Val Val Tyr Ile Tyr Glu Asn Ala Thr His Leu Asp Glu Val Val Thr Leu Ile Ala Ser Asp Leu Asp Arg

```
2930        2940        2950        2960        2970        2980        2990        3000        3010
GAC GAA ATA TAC CAC ACG GTG AGC TAC GTC ATC AAT TAT GCA GTG AAC CCT CGA CTG ATG AAC TTC TTC TCC GTG AAC CGA GAG ACC GGC
Asp Glu Ile Tyr His Thr Val Ser Tyr Val Ile Asn Tyr Ala Val Asn Pro Arg Leu Met Asn Phe Phe Ser Val Asn Arg Glu Thr Gly

3020        3030        3040        3050        3060        3070        3080        3090        3100
CTG GTG TAC GTG GAC TAT GAG ACC CAG GGT AGT GGC GAG GTG CTG GAC CGT GAT GGT GAT GAA CCA ACG CAC CGT ATC TTC TTC AAC CTC
Leu Val Tyr Val Asp Tyr Glu Thr Gln Gly Ser Gly Glu Val Leu Asp Arg Asp Gly Asp Glu Pro Thr His Arg Ile Phe Phe Asn Leu

3110        3120        3130        3140        3150        3160        3170        3180        3190
ATC GAC AAC TTC ATG GGG GAA GGA GAA GGT AAC AGA AAT CAG AAC GAC ACA GAA GTT CTC GTT ATC TTG TTG GAT CTG AAT GAC AAT GCT
Ile Asp Asn Phe Met Gly Glu Gly Glu Gly Asn Arg Asn Gln Asn Asp Thr Glu Val Leu Val Ile Leu Leu Asp Val Asn Asp Asn Ala

3200        3210        3220        3230        3240        3250        3260        3270        3280
CCT GAA TTG CCA CCG CCG AGC GAA CTC TCT TGG ACT ATA TCT GAG AAC CTT AAG CAG GGC GTC CGT CTT GAA CCA CAT ATC TTC GCC CCG
Pro Glu Leu Pro Pro Pro Ser Glu Leu Ser Trp Thr Ile Ser Glu Asn Leu Lys Gln Gly Val Arg Leu Glu Pro His Ile Phe Ala Pro

3290        3300        3310        3320        3330        3340        3350        3360        3370
GAC CGC GAC GAG CCC GAC ACA GAC AAC TCC AGG GTC GGC TAC GAG ATC CTG AAC CTC AGC ACG GAG CGG GAC ATC GAA GTG CCG GAG CTG
Asp Arg Asp Glu Pro Asp Thr Asp Asn Ser Arg Val Gly Tyr Glu Ile Leu Asn Leu Ser Thr Glu Arg Asp Ile Glu Val Pro Glu Leu

3380        3390        3400        3410        3420        3430        3440        3450        3460
TTT GTG ATG ATA CAG ATC GCG AAC GTC ACG GGA GAG CTG GAG ACC GCC ATG GAC CTC AAG GGA TAT TGG GGG ACG TAC GCT ATA CAT ATA
Phe Val Met Ile Gln Ile Ala Asn Val Thr Gly Glu Leu Glu Thr Ala Met Asp Leu Lys Gly Tyr Trp Gly Thr Tyr Ala Ile His Ile

3470        3480        3490        3500        3510        3520        3530        3540        3550
CGG GCA TTC GAC CAC GGC ATT CCG CAA ATG TCC ATG AAC GAG ACA TAT GAG CTG ATC ATC CAT CCG TTC AAC TAC TAC GCG CCT GAG TTC
Xaa Ala Phe Asp His Gly Ile Pro Gln Met Ser Met Asn Glu Thr Tyr Glu Leu Ile Ile His Pro Phe Asn Tyr Tyr Ala Pro Glu Phe

3560        3570        3580        3590        3600        3610        3620        3630        3640
GTC TTC CCG ACC AAC GAT GCC GTC ATA CGA CTT GCG AGG GAA CGA GCT GTA ATC AAT GGA GTT CTA GCG ACA GTG AAC GGA GAG TTC TTG
Val Phe Pro Thr Asn Asp Ala Val Ile Arg Leu Ala Arg Glu Arg Ala Val Ile Asn Gly Val Leu Ala Thr Val Asn Gly Glu Phe Leu

3650        3660        3670        3680        3690        3700        3710        3720        3730
GAG CGG ATA TCG GCG ACT GAT CCG GAC GGA CTC CAC GCG GGC GTC GTC ACC TTC CAA GTG GTA GGC GAT GAG GAA TCA CAA CGG TAC TTT
Glu Arg Ile Ser Ala Thr Asp Pro Asp Gly Leu His Ala Gly Val Val Thr Phe Gln Val Val Gly Asp Glu Glu Ser Gln Arg Tyr Phe

3740        3750        3760        3770        3780        3790        3800        3810        3820
CAA GTA GTT AAC GAT GGC GAG AAC CTC GGC TCG TTG AGG TTA CTG CAA GCC GTT CCA GAG GAG ATC AGG GAG TTC CGG ATA ACG ATT CGC
Gln Val Val Asn Asp Gly Glu Asn Leu Gly Ser Leu Arg Leu Leu Gln Ala Val Pro Glu Glu Ile Arg Glu Phe Arg Ile Thr Ile Arg

3830        3840        3850        3860        3870        3880        3890        3900        3910
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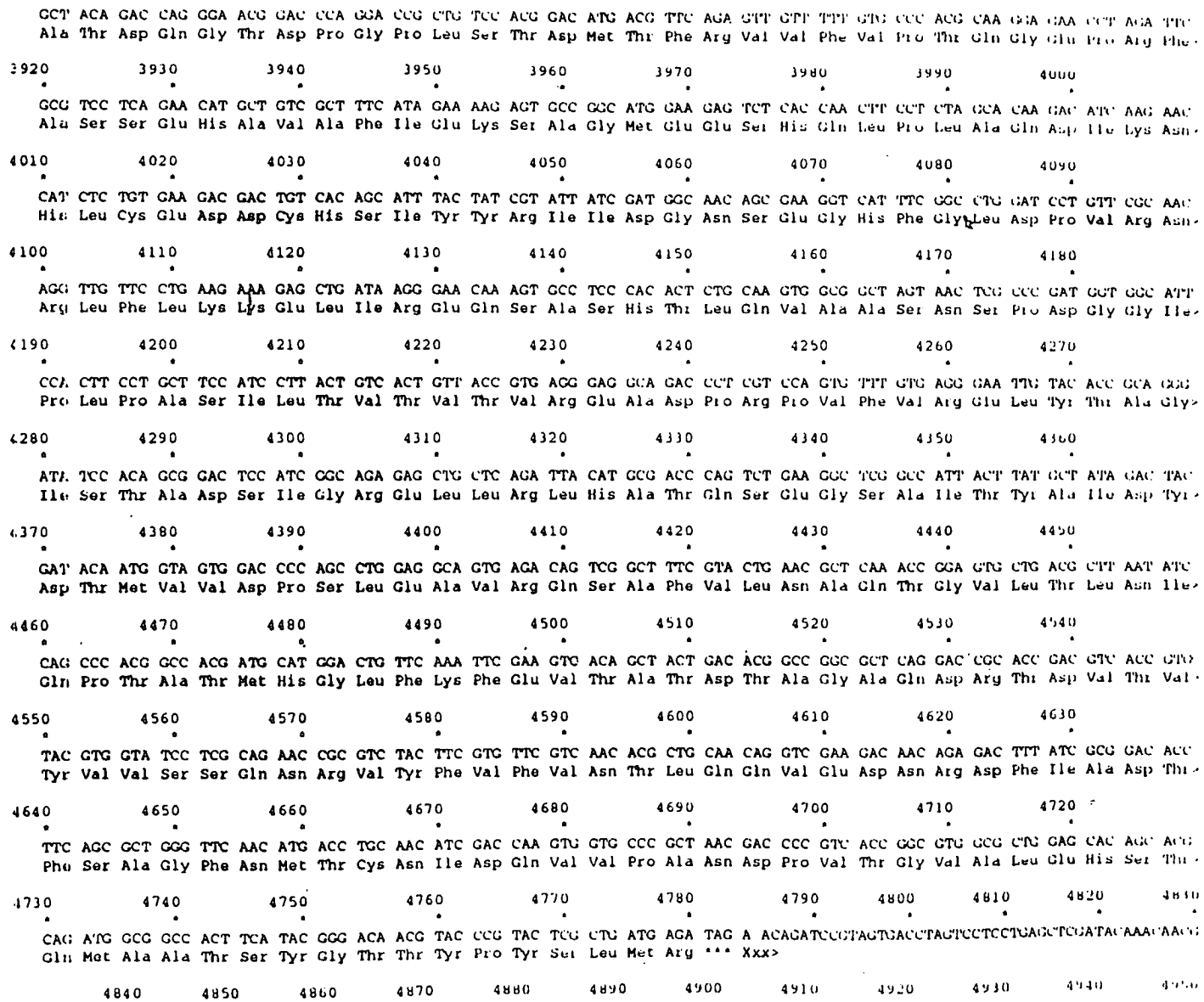
Fig 1(cont)



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   Ala Thr Asp Gln Gly Thr Asp Pro Gly Pro Leu Ser Thr Asp Met Thr Phe Arg Val Val Phe Val Pro Thr Gln Gly Glu Pro Arg Phe>

3920        3930        3940        3950        3960        3970        3980        3990        4000
   .           .           .           .           .           .           .           .           .
   GCG TCC TCA GAA CAT GCT GTC GCT TTC ATA GAA AAG AGT GCC GGC ATG GAA GAG TCT CAC CAA CTT CCT CTA GCA CAA GAC ATC AAG AAC
   Ala Ser Ser Glu His Ala Val Ala Phe Ile Glu Lys Ser Ala Gly Met Glu Glu Ser His Gln Leu Pro Leu Ala Gln Asp Ile Lys Asn>

4010        4020        4030        4040        4050        4060        4070        4080        4090
   .           .           .           .           .           .           .           .           .
   CAT CTC TGT GAA GAC GAC TGT CAC AGC ATT TAC TAT CGT ATT ATC GAT GGC AAC AGC GAA GGT CAT TTC GGC CTG GAT CCT GTT CGC AAC
   His Leu Cys Glu Asp Asp Cys His Ser Ile Tyr Tyr Arg Ile Ile Asp Gly Asn Ser Glu Gly His Phe Gly Leu Asp Pro Val Arg Asn>

4100        4110        4120        4130        4140        4150        4160        4170        4180
   .           .           .           .           .           .           .           .           .
   AGG TTG TTC CTG AAG AAA GAG CTG ATA AGG GAA CAA AGT GCC TCC CAC ACT CTG CAA GTG GCG GCT AGT AAC TCG CCC GAT GGT GGC ATT
   Arg Leu Phe Leu Lys Lys Glu Leu Ile Arg Glu Gln Ser Ala Ser His Thr Leu Gln Val Ala Ala Ser Asn Ser Pro Asp Gly Gly Ile>

4190        4200        4210        4220        4230        4240        4250        4260        4270
   .           .           .           .           .           .           .           .           .
   CCA CTT CCT GCT TCC ATC CTT ACT GTC ACT GTT ACC GTG AGG GAG GCA GAC CCT CGT CCA GTG TTT GTG AGG GAA TTG TAC ACC GCA GGC
   Pro Leu Pro Ala Ser Ile Leu Thr Val Thr Val Thr Val Arg Glu Ala Asp Pro Arg Pro Val Phe Val Arg Glu Leu Tyr Thr Ala Gly>

4280        4290        4300        4310        4320        4330        4340        4350        4360
   .           .           .           .           .           .           .           .           .
   ATA TCC ACA GCG GAC TCC ATC GGC AGA GAG CTG CTC AGA TTA CAT GCG ACC CAG TCT GAA GGC TCG GCC ATT ACT TAT GCT ATA GAC TAC
   Ile Ser Thr Ala Asp Ser Ile Gly Arg Glu Leu Leu Arg Leu His Ala Thr Gln Ser Glu Gly Ser Ala Ile Thr Tyr Ala Ile Asp Tyr>

4370        4380        4390        4400        4410        4420        4430        4440        4450
   .           .           .           .           .           .           .           .           .
   GAT ACA ATG GTA GTG GAC CCC AGC CTG GAG GCA GTG AGA CAG TCG GCT TTC GTA CTG AAC GCT CAA ACC GGA GTG CTG ACG CTT AAT ATC
   Asp Thr Met Val Val Asp Pro Ser Leu Glu Ala Val Arg Gln Ser Ala Phe Val Leu Asn Ala Gln Thr Gly Val Leu Thr Leu Asn Ile>

4460        4470        4480        4490        4500        4510        4520        4530        4540
   .           .           .           .           .           .           .           .           .
   CAG CCC ACG GCC ACG ATG CAT GGA CTG TTC AAA TTC GAA GTG ACA GCT ACT GAC ACG GCC GGC GCT CAG GAC CGC ACC GAC GTC ACC GTG
   Gln Pro Thr Ala Thr Met His Gly Leu Phe Lys Phe Glu Val Thr Ala Thr Asp Thr Ala Gly Ala Gln Asp Arg Thr Asp Val Thr Val>

4550        4560        4570        4580        4590        4600        4610        4620        4630
   .           .           .           .           .           .           .           .           .
   TAC GTG GTA TCC TCG CAG AAC CGC GTC TAC TTC GTG TTC GTC AAC ACG CTG CAA CAG GTC GAA GAC AAC AGA GAC TTT ATC GCG GAC ACC
   Tyr Val Val Ser Ser Gln Asn Arg Val Tyr Phe Val Phe Val Asn Thr Leu Gln Gln Val Glu Asp Asn Arg Asp Phe Ile Ala Asp Thr>

4640        4650        4660        4670        4680        4690        4700        4710        4720
   .           .           .           .           .           .           .           .           .
   TTC AGC GCT GGG TTC AAC ATG ACC TGC AAC ATC GAC CAA GTG GTG CCC GCT AAC GAC CCC GTC ACC GGC GTG GCG CTG GAG CAC AGC ACG
   Phe Ser Ala Gly Phe Asn Met Thr Cys Asn Ile Asp Gln Val Val Pro Ala Asn Asp Pro Val Thr Gly Val Ala Leu Glu His Ser Thr>

4730        4740        4750        4760        4770        4780        4790        4800        4810        4820        4830
   .           .           .           .           .           .           .           .           .           .           .
   CAG ATG GCG GCC ACT TCA TAC GGG ACA ACG TAC CCG TAC TCG CTG ATG AGA TAG A ACAGATCCGTAGTGACCTAGTCCTCCTCAGCTCGATACAAACAACG
   Gln Met Ala Ala Thr Ser Tyr Gly Thr Thr Tyr Pro Tyr Ser Leu Met Arg *** Xxx>

        4840        4850        4860        4870        4880        4890        4900        4910        4920        4930        4940        4950
```

Fig. 1A

CTGGCGGCGCGATCGTGGTGTTGCAGGACTTGTTGACCAACTCCAGCCCGGACTTCGGCGCCTGACTCGAGCCTGCACGGTGTACCTCTGGCCTCACTGTCTGCTGTGCTCGGTTTCATG

4960 4970 4980 4990 5000 5010 5020 5030 5040 5050 5060 5070

TGCCTTGTGCTACTGCTTACCTTCATCATCAGGACTAC.. GCTAAACCGACGGTTGGAAGCCCTGTCGATGACGAAGTACGGCTCACTGGACTCTGGATTGAACCGCGCCGGCATCGCC

5080 5090 5100 5110 5120 5130 5140 5150 5160 5170 5180 5190

GCCCCCGGCACCAACAAACACACTGTGGAAGGCTCCAACCCTATCTTCAATGAAGCAATAAAGACGCCAGATTTAGATGCCATTAGCGAGGTTCCAACGACTCTGATCTGATCGGCATC

5200 5210 5220 5230 5240 5250 5260 5270 5280 5290 5300 5310

GAAGATCTTGCGCACTTTGGCAACGTCTTCATGGATCCTGAGGTGAACGAAAAGGCAAATGGTTATCCCGAAGTCGCAAACCACAACAACAACTTCGCTTTCAACCCGACTCCCTTCTCG

5320 5330 5340 5350 5360 5370 5380 5390 5400 5410 5420 5430

CCTCAGTTCGTTAACGGACAGTTCAGAAAGATCTAGAAGATAACAACACTAGTTAAGATCATTAATTTTGGAGTTTGGAATTAAGATTTTTGAAAGGATAGTTGTGATAACCCTGTGATT

5440 5450 5460 5470 5480 5490 5500 5510 5520 5530 5540 5550

TTTAAAACTGTAATTGAAAAAAAAAATTGAGACCTCCATTTAAGCTCTTGCTCTCATCTCATCAAATTTTATAAAATGCCATTAGTCATTAAGATACTCGATTTAATTTAAGATTATTTA

5560 5570

AGATATTATGTAAAATAAATATATTGTC

KEY TO UPDATED SEQUENCE:

updated nucleotide number:	type of change:
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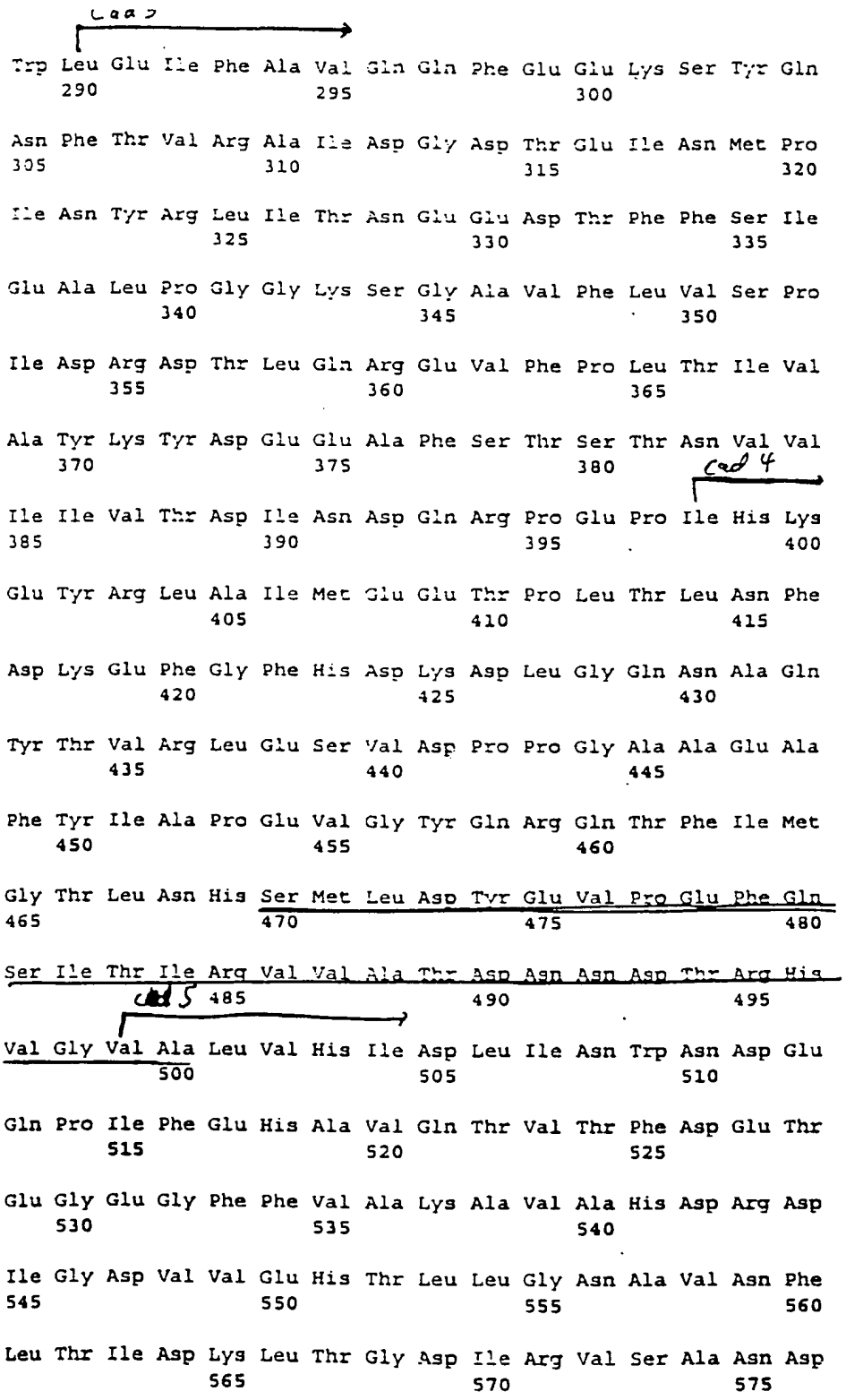
2105-08	rearrangement
2627-8	deletion
2668	'C' addition
2678	'G' addition
2946	rearrangement
3464	rearrangement
3471	rearrangement
4835-6	deletion
4884-4892	addition
4938	addition
5030	deletion
5031	rearrangement
5200	rearrangement
5216	rearrangement
5288	deletion
5422	rearrangement

Fig. 1 (Con't)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Ile Asn Tyr Arg Leu Ile Thr Asn Glu Glu Asp Thr Phe Phe Ser Ile
325 330 335
Glu Ala Leu Pro Gly Gly Lys Ser Gly Ala Val Phe Leu Val Ser Pro
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Ala Tyr Lys Tyr Asp Glu Glu Ala Phe Ser Thr Ser Thr Asn Val Val
370 375 380
Cad 4
Ile Ile Val Thr Asp Ile Asn Asp Gln Arg Pro Glu Pro Ile His Lys
385 390 395 400
Glu Tyr Arg Leu Ala Ile Met Glu Glu Thr Pro Leu Thr Leu Asn Phe
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Asp Lys Glu Phe Gly Phe His Asp Lys Asp Leu Gly Gln Asn Ala Gln
420 425 430
Tyr Thr Val Arg Leu Glu Ser Val Asp Pro Pro Gly Ala Ala Glu Ala
435 440 445
Phe Tyr Ile Ala Pro Glu Val Gly Tyr Gln Arg Gln Thr Phe Ile Met
450 455 460
Gly Thr Leu Asn His Ser Met Leu Asp Tyr Glu Val Pro Glu Phe Gln
465 470 475 480
Ser Ile Thr Ile Arg Val Val Ala Thr Asp Asn Asn Asp Thr Arg His
Cad 5
485 490 495
Val Gly Val Ala Leu Val His Ile Asp Leu Ile Asn Trp Asn Asp Glu
500 505 510
Gln Pro Ile Phe Glu His Ala Val Gln Thr Val Thr Phe Asp Glu Thr
515 520 525
Glu Gly Glu Gly Phe Phe Val Ala Lys Ala Val Ala His Asp Arg Asp
530 535 540
Ile Gly Asp Val Val Glu His Thr Leu Leu Gly Asn Ala Val Asn Phe
545 550 555 560
Leu Thr Ile Asp Lys Leu Thr Gly Asp Ile Arg Val Ser Ala Asn Asp
565 570 575

FIGURE 2

```
Ser Phe Asn Tyr His Arg Glu Ser Glu Leu Phe Val Gln Val Arg Ala
            580                 585                 590

Thr Asp Thr Leu Gly Glu Pro Phe His Thr Ala Thr Ser Gln Leu Val
        595                 600                 605

Ile Arg Leu Asn Asp Ile Asn Asn Thr Pro Pro Thr Leu Arg Leu Pro
    610                 615                 620

Arg Gly Ser Pro Gln Val Glu Glu Asn Val Pro Asp Gly His Val Ile
625                 630                 635                 640

Thr Gln Glu Leu Arg Ala Thr Asp Pro Asp Thr Thr Ala Asp Leu Arg
                645                 650                 655

Phe Glu Ile Asn Trp Asp Thr Ser Phe Ala Thr Lys Gln Gly Arg Gln
            660                 665                 670

Ala Asn Pro Asp Glu Phe Arg Asn Cys Val Glu Ile Glu Thr Ile Phe
        675                 680                 685

Pro Glu Ile Asn Asn Arg Gly Leu Ala Ile Gly Arg Val Val Ala Arg
    690                 695                 700

Glu Ile Arg His Asn Val Thr Ile Asp Tyr Glu Glu Phe Glu Val Leu
705                 710                 715                 720

Ser Leu Thr Val Arg Val Arg Asp Leu Asn Thr Val Tyr Gly Asp Asp
                725                 730                 735

Tyr Asp Glu Ser Met Leu Thr Ile Thr Ile Ile Asp Met Asn Asp Asn
            740                 745                 750

Ala Pro Val Trp Val Glu Gly Thr Leu Glu Gln Asn Phe Arg Val Arg
        755                 760                 765

Glu Met Ser Ala Gly Gly Leu Val Val Gly Ser Val Arg Ala Asp Asp
    770                 775                 780

Ile Asp Gly Pro Leu Tyr Asn Gln Val Arg Tyr Thr Ile Phe Pro Arg
785                 790                 795                 800

Glu Asp Thr Asp Lys Asp Leu Ile Met Ile Asp Phe Leu Thr Gly Gln
                805                 810                 815

Ile Ser Val Asn Thr Ser Gly Ala Ile Asp Ala Asp Thr Pro Pro Arg
            820                 825                 830

Phe His Leu Tyr Tyr Thr Val Val Ala Ser Asp Arg Cys Ser Thr Glu
        835                 840                 845

Asp Pro Ala Asp Cys Pro Pro Asp Pro Thr Tyr Trp Glu Thr Glu Gly
    850                 855                 860
```

FIGURE 2

```
Asn Ile Thr Ile His Ile Thr Asp Thr Asn Asn Lys Val Pro Gln Ala
865                 870                 875                 880

Glu Thr Thr Lys Phe Asp Thr Val Val Tyr Ile Tyr Glu Asn Ala Thr
                885                 890                 895

His Leu Asp Glu Val Val Thr Leu Ile Ala Ser Asp Leu Asp Arg Asp
            900                 905                 910

Glu Ile Tyr His Thr Val Ser Tyr Val Ile Asn Tyr Ala Val Asn Pro
        915                 920                 925

Arg Leu Met Asn Phe Phe Ser Val Asn Arg Glu Thr Gly Leu Val Tyr
    930                 935                 940

Val Asp Tyr Glu Thr Gln Gly Ser Gly Glu Val Leu Asp Arg Asp Gly
945                 950                 955                 960

Asp Glu Pro Thr His Arg Ile Phe Phe Asn Leu Ile Asp Asn Phe Met
                965                 970                 975

Gly Glu Gly Glu Gly Asn Arg Asn Gln Asn Asp Thr Glu Val Leu Val
            980                 985                 990

Ile Leu Leu Asp Val Asn Asp Asn Ala Pro Glu Leu Pro Pro Pro Ser
        995                 1000                1005

Glu Leu Ser Trp Thr Ile Ser Glu Asn Leu Lys Gln Gly Val Arg Leu
    1010                1015                1020

Glu Pro His Ile Phe Ala Pro Asp Arg Asp Glu Pro Asp Thr Asp Asn
1025                1030                1035                1040

Ser Arg Val Gly Tyr Glu Ile Leu Asn Leu Ser Thr Glu Arg Asp Ile
                1045                1050                1055

Glu Val Pro Glu Leu Phe Val Met Ile Gln Ile Ala Asn Val Thr Gly
            1060                1065                1070

Glu Leu Glu Thr Ala Met Asp Leu Lys Gly Tyr Trp Gly Thr Tyr Ala
        1075                1080                1085

Ile His Ile Arg Ala Phe Asp His Gly Ile Pro Gln Met Ser Met Asn
    1090                1095                1100

Glu Thr Tyr Glu Leu Ile Ile His Pro Phe Asn Tyr Tyr Ala Pro Glu
1105                1110                1115                1120

Phe Val Phe Pro Thr Asn Asp Ala Val Ile Arg Leu Ala Arg Glu Arg
                1125                1130                1135

Ala Val Ile Asn Gly Val Leu Ala Thr Val Asn Gly Glu Phe Leu Glu
            1140                1145                1150
```

FIGURE 2

```
Arg Ile Ser Ala Thr Asp Pro Asp Gly Leu His Ala Gly Val Val Thr
        1155                1160                1165

Phe Gln Val Val Gly Asp Glu Glu Ser Gln Arg Tyr Phe Gln Val Val
    1170                1175                1180

Asn Asp Gly Glu Asn Leu Gly Ser Leu Arg Leu Leu Gln Ala Val Pro
1185                1190                1195                1200

Glu Glu Ile Arg Glu Phe Arg Ile Thr Ile Arg Ala Thr Asp Gln Gly
                1205                1210                1215

Thr Asp Pro Gly Pro Leu Ser Thr Asp Met Thr Phe Arg Val Val Phe
            1220                1225                1230

Val Pro Thr Gln Gly Glu Pro Arg Phe Ala Ser Ser Glu His Ala Val
        1235                1240                1245

Ala Phe Ile Glu Lys Ser Ala Gly Met Glu Glu Ser His Gln Leu Pro
    1250                1255                1260

Leu Ala Gln Asp Ile Lys Asn His Leu Cys Glu Asp Asp Cys His Ser
1265                1270                1275                1280

Ile Tyr Tyr Arg Ile Ile Asp Gly Asn Ser Glu Gly His Phe Gly Leu
                1285                1290                1295

Asp Pro Val Arg Asn Arg Leu Phe Leu Lys Lys Glu Leu Ile Arg Glu
            1300                1305                1310

Gln Ser Ala Ser His Thr Leu Gln Val Ala Ala Ser Asn Ser Pro Asp
        1315                1320                1325

Gly Gly Ile Pro Leu Pro Ala Ser Ile Leu Thr Val Thr Val Thr Val
    1330                1335                1340

Arg Glu Ala Asp Pro Arg Pro Val Phe Val Arg Glu Leu Tyr Thr Ala
1345                1350                1355                1360

Gly Ile Ser Thr Ala Asp Ser Ile Gly Arg Glu Leu Leu Arg Leu His
                1365                1370                1375

Ala Thr Gln Ser Glu Gly Ser Ala Ile Thr Tyr Ala Ile Asp Tyr Asp
            1380                1385                1390

Thr Met Val Val Asp Pro Ser Leu Glu Ala Val Arg Gln Ser Ala Phe
        1395                1400                1405

Val Leu Asn Ala Gln Thr Gly Val Leu Thr Leu Asn Ile Gln Pro Thr
    1410                1415                1420

Ala Thr Met His Gly Leu Phe Lys Phe Glu Val Thr Ala Thr Asp Thr
1425                1430                1435                1440
```

FIGURE 2

```
Ala Gly Ala Gln Asp Arg Thr Asp Val Thr Val Tyr Val Val Ser Ser
                1445                1450                1455

Gln Asn Arg Val Tyr Phe Val Phe Val Asn Thr Leu Gln Gln Val Glu
            1460                1465                1470

Asp Asn Arg Asp Phe Ile Ala Asp Thr Phe Ser Ala Gly Phe Asn Met
        1475                1480                1485

Thr Cys Asn Ile Asp Gln Val Val Pro Ala Asn Asp Pro Val Thr Gly
    1490                1495                1500

Val Ala Leu Glu His Ser Thr Gln Met Ala Ala Thr Ser Tyr Gly Thr
1505                1510                1515                1520

Thr Tyr Pro Tyr Ser Leu Met Arg
                1525
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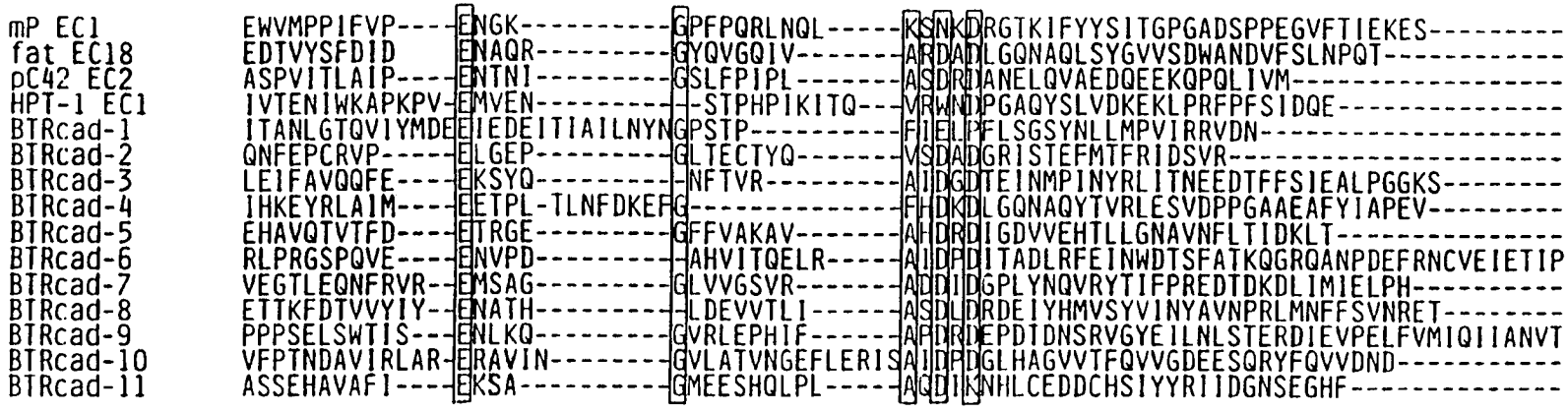
FIGURE 2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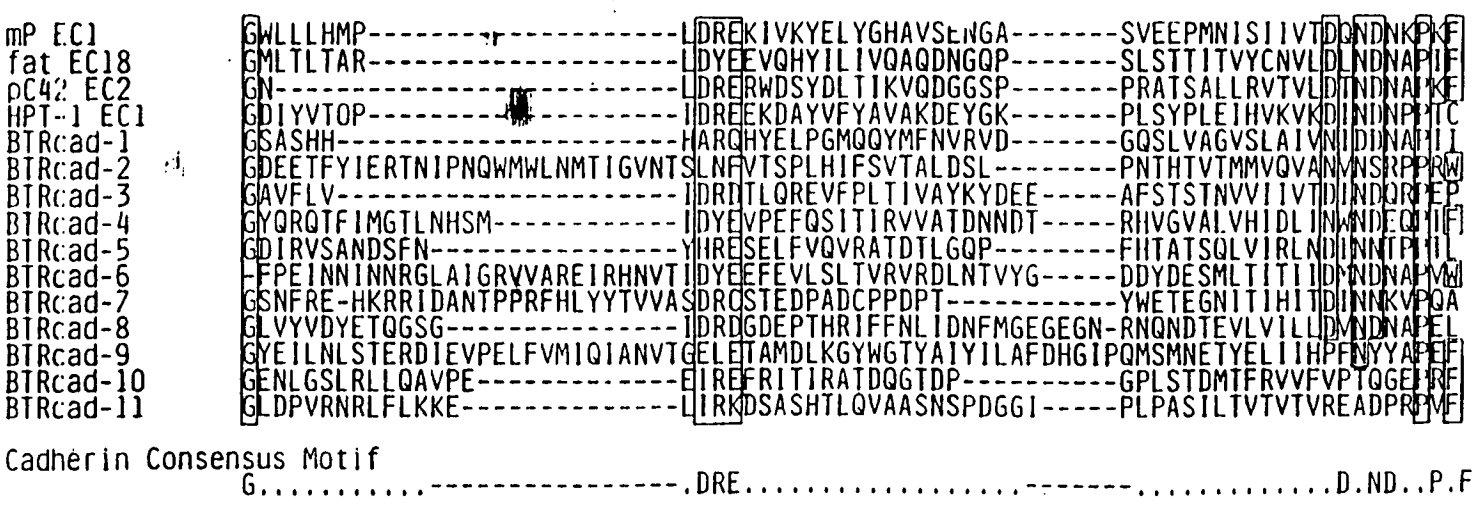
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pC42 EC2 GN----------LDRERWDSYDLTIKVQDGGSP-------PRATSALLRVTVLDTNDNAIKE
HPT-1 EC1 GDIYVTOP----------IDREEKDAYVFYAVAKDEYGK-------PLSYPLEIHVKVKDINDNPPTC
BTRcad-1 GSASHH----------HARQHYELPGMQQYMFNVRVD-------GQSLVAGVSLAIVNIDDNAPII
BTRcad-2 GDEETFYIERTNIPNQWMWLNMTIGVNTSLNFVTSPLHIFSVTALDSL--------PNTHTVTMMVQVANMNSRPPRW
BTRcad-3 GAVFLV----------IDRDTLQREVFPLTIVAYKYDEE-----AFSTSTNVVIIVTDINDQRPEP
BTRcad-4 GYQRQTFIMGTLNHSM----------IDYEVPEFQSITIRVVATDNNDT-----RHVGVALVHIDLINWNDEQPIF
BTRcad-5 GDIRVSANDSFN----------YHRESELFVQVRATDTLGQP--------FHTATSQLVIRLNDINNTPPIL
BTRcad-6 FPEINNINNRGLAIGRVVAREIRHNVTIDYEEFEVLSLTVRVRDLNTVYG-----DDYDESMLTITIIDMNDNAPVW
BTRcad-7 GSNFRE-HKRRIDANTPPRFHLYYTVVASDRGSTEDPADCPPDPT----------YWETEGNITIHITDINNKVPQA
BTRcad-8 GLVYVDYETQGSG----------IDRDGDEPTHRIFFNLIDNFMGEGEGN-RNQNDTEVLVILLDMNDNAPEL
BTRcad-9 GYEILNLSTERDIEVPELFVMIQIANVTGELETAMDLKGYWGTYAIYILAFDHGIPQMSMNETYELIIHPFNYYAPEF
BTRcad-10 GENLGSLRLLQAVPE----------EIREFRITIRATDQGTDP----------GPLSTDMTFRVVFVPTQGEFRF
BTRcad-11 GLDPVRNRLFLKKE----------LIRKDSASHTLQVAASNSPDGGI-----PLPASILTVTVTVREADPRPVF
Cadherin Consensus Motif
G.....----------.DRE----------.....D.ND..P.F

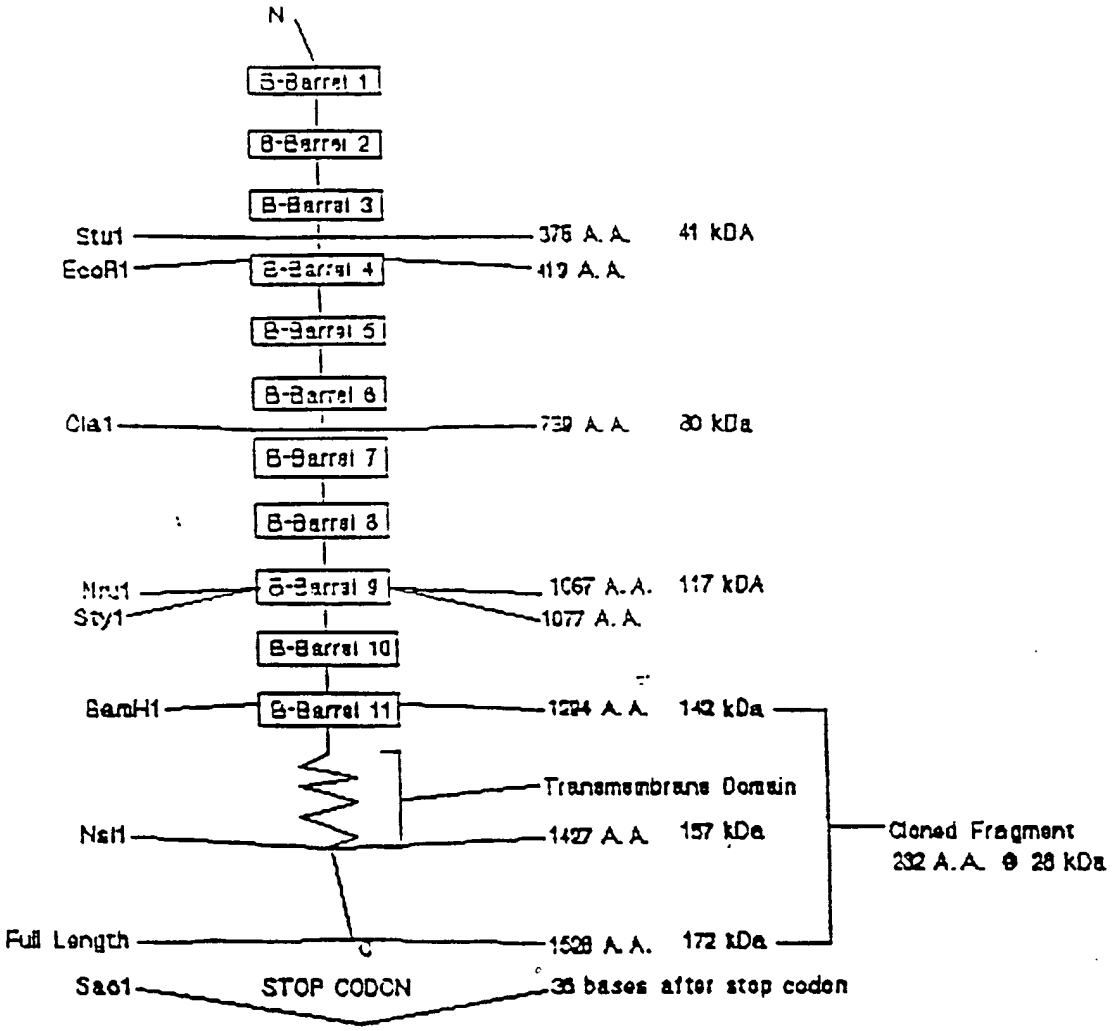

Fig. 3 Block diagram of cadherin-like structure of BT-R_1

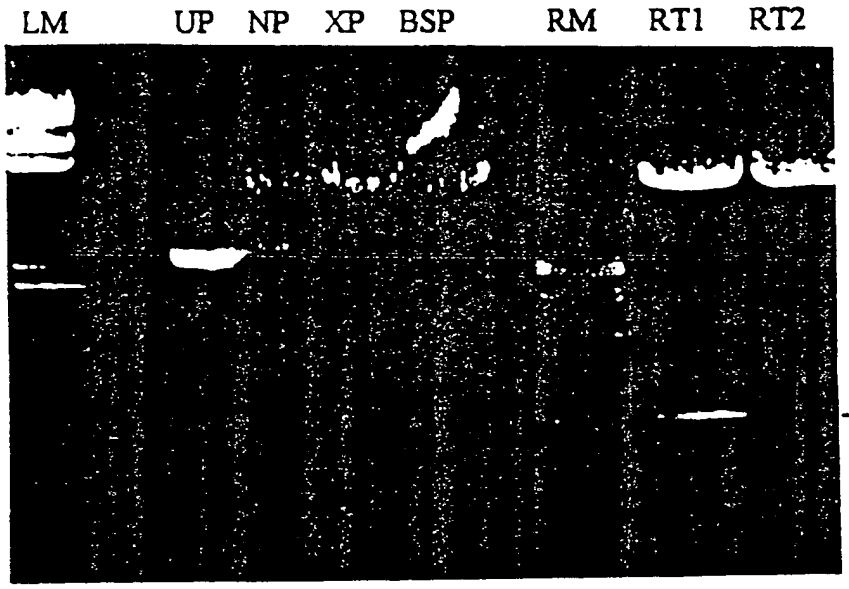

Fig. 4 Clone characterization of BamHI-SacI fragment of BT-R_1. LM is HindIII cut Lambda marker; UP is the uncut plasmid clone; NP is NsiI cut plasmid; XP is XhoI cut plasmid; BSP is BamHI and SacI cut plasmid showing the cloned fragment from BT-R_1; RM is mRNA size marker; and RT1 and RT2 are transcribed mRNAs from the cloned BT-R_1 fragment.

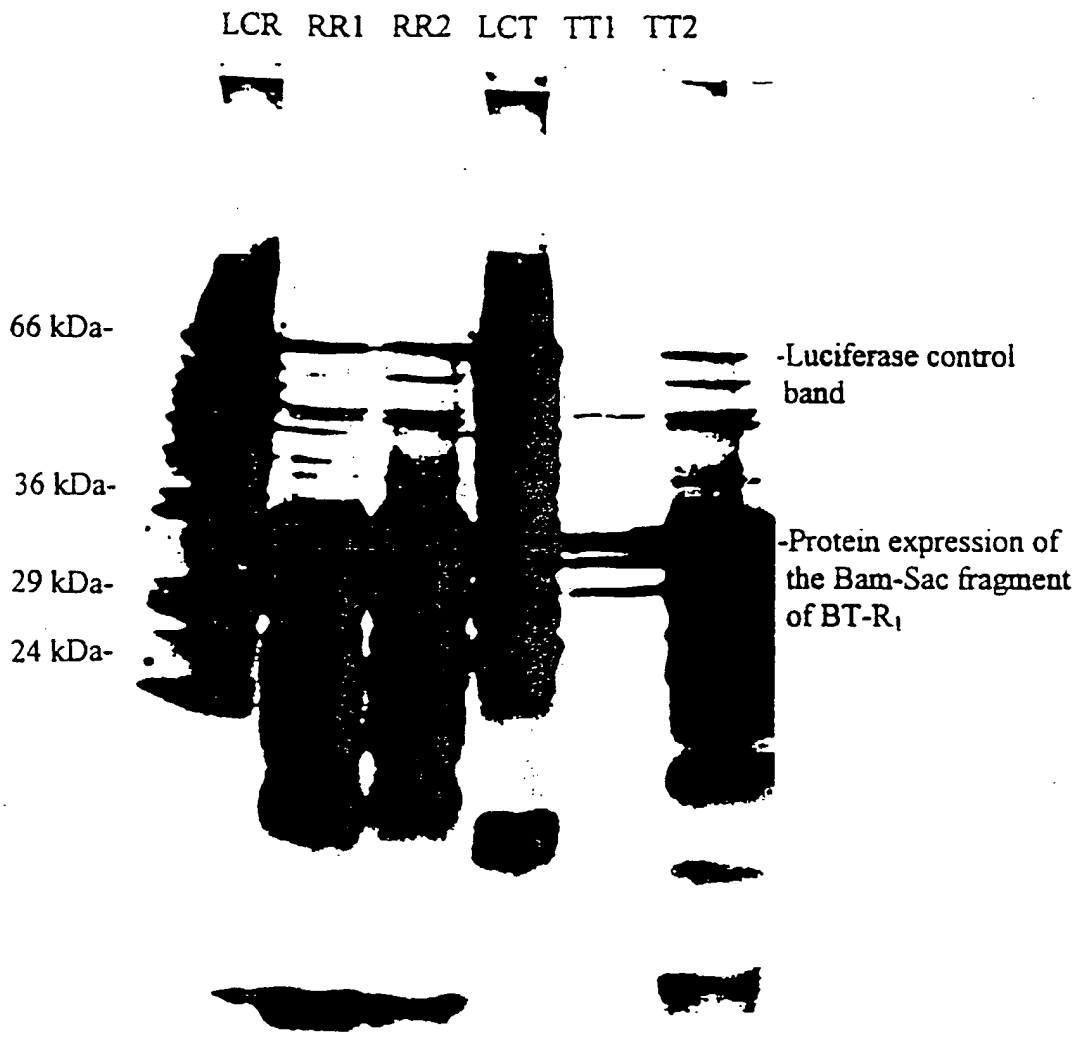

Fig. 5 Detection of protein expression from the plasmid containing the Bam-Sac fragment of BT-R_1 using ^{35}S-methionine as a tag. LCR is a luciferase control mRNA to show that the rabbit reticulocyte lysates are functional; RR1 and RR2 are expression products of the Bam-Sac fragment of BT-R_1 prodced in rabbit reticulocytes from mRNA; LCT is a luciferase control plasmid to show that the transcription/translation kit is functional; and TT1 and TT2 are expression products of the Bam-Sac fragment of BT-R_1 produced in a transcription/translation kit.

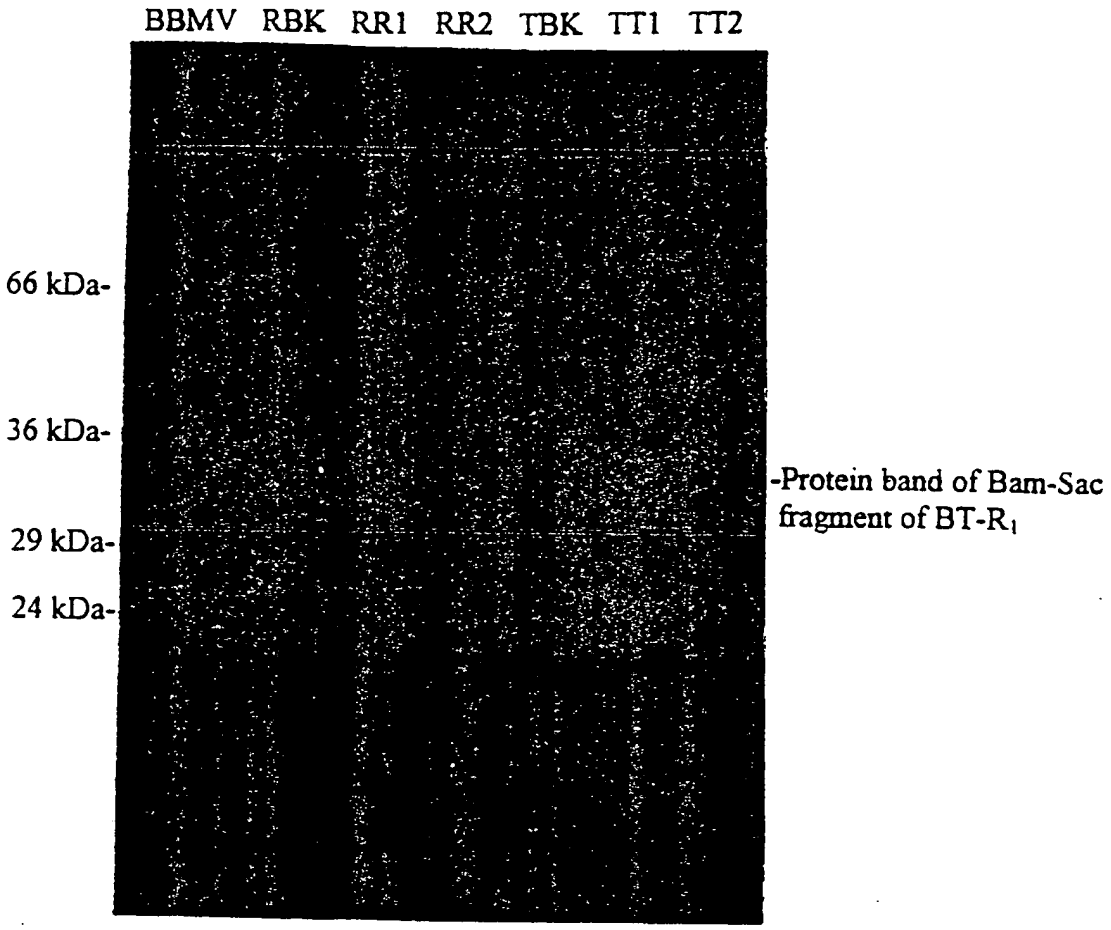

Fig. 6 Radio-blot of the Bam-Sac fragment of BT-R_1 with ^{125}I-labeled Cry1Ab. BBMV is the brush border membrane vesicles from the midgut of *M. Sexta* containing the wild-type BT-R_1 receptor protein;RBK is a rabbit reticulocyte blank; RR1 and RR2 are the expression products of the Bam-Sac fragment of BT-R_1 prodced in rabbit reticulocytes from mRNA; TBK is a transcription/translation kit blank; TT1 and TT2 are expression products of the Bam-Sac fragment of BT-R_1 produced in a transcription/translation kit. The arrows point to two of the bands.

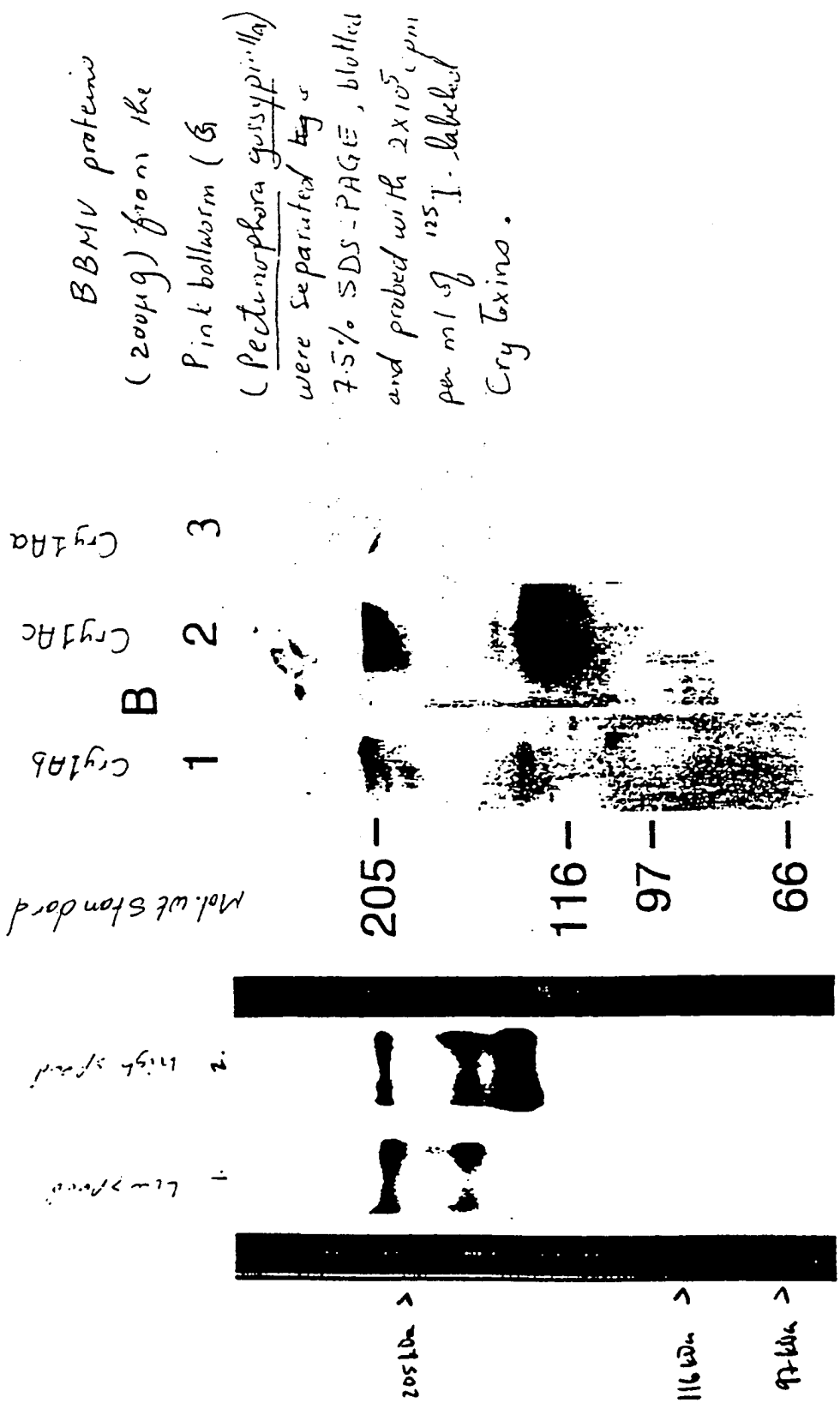
BBMV proteins (200µg) from the Pink bollworm (Pectinophora gossypiella) were separated by 7.5% SDS-PAGE, blotted and probed with 2x10^5 cpm per ml of 125I-labelled Cry Toxins.
Cry1Ab
Cry1Ac
Cry1Aa
B
1
2
3
Mol.wt Standard
205
116
97
66
205 kDa
116 kDa
97 kDa
66 kDa

Fig 7

```
Met Ala Val Asp Val Arg Ile Ala Ala Phe Leu Leu Val Phe Ile Ala
 1               5                  10                  15

Pro Ala Val Leu Ala Gln Glu Arg Cys Gly Tyr Met Thr Ala Ile Pro
            20                  25                  30

Arg Leu Pro Arg Pro Asp Asn Leu Pro Val Leu Asn Phe Glu Gly Gln
        35                  40                  45

Thr Trp Ser Gln Arg Pro Leu Leu Pro Ala Pro Glu Arg Asp Asp Leu
    50                  55  Cad1            60

Cys Met Asp Ala Tyr His Val Ile Thr Ala Asn Leu Gly Thr Gln Val
65                  70                  75                  80

Ile Tyr Met Asp Glu Glu Ile Glu Asp Glu Ile Thr Ile Ala Ile Leu
                85                  90                  95

Asn Tyr Asn Gly Pro Ser Thr Pro Phe Ile Glu Leu Pro Phe Leu Ser
            100                 105                 110

Gly Ser Tyr Asn Leu Leu Met Pro Val Ile Arg Arg Val Asp Asn Gly
        115                 120                 125

Ser Ala Ser His His His Ala Arg Gln His Tyr Glu Leu Pro Gly Met
    130                 135                 140

Gln Gln Tyr Met Phe Asn Val Arg Val Asp Gly Gln Ser Leu Val Ala
145                 150                 155                 160

Gly Val Ser Leu Ala Ile Val Asn Ile Asp Asp Asn Ala Pro Ile Ile
    Cad2        165                 170                 175

Gln Asn Phe Glu Pro Cys Arg Val Pro Glu Leu Gly Glu Pro Gly Leu
            180                 185                 190

Thr Glu Cys Thr Tyr Gln Val Ser Asp Ala Asp Gly Arg Ile Ser Thr
        195                 200                 205

Glu Phe Met Thr Phe Arg Ile Asp Ser Val Arg Gly Asp Glu Glu Thr
    210                 215                 220

Phe Tyr Ile Glu Arg Thr Asn Ile Pro Asn Gln Trp Met Trp Leu Asn
225                 230                 235                 240

Met Thr Ile Gly Val Asn Thr Ser Leu Asn Phe Val Thr Ser Pro Leu
                245                 250                 255

His Ile Phe Ser Val Thr Ala Leu Asp Ser Leu Pro Asn Thr His Thr
            260                 265                 270

Val Thr Met Met Val Gln Val Ala Asn Val Asn Ser Arg Pro Pro Arg
        275                 280                 285
```

FIGURE 2

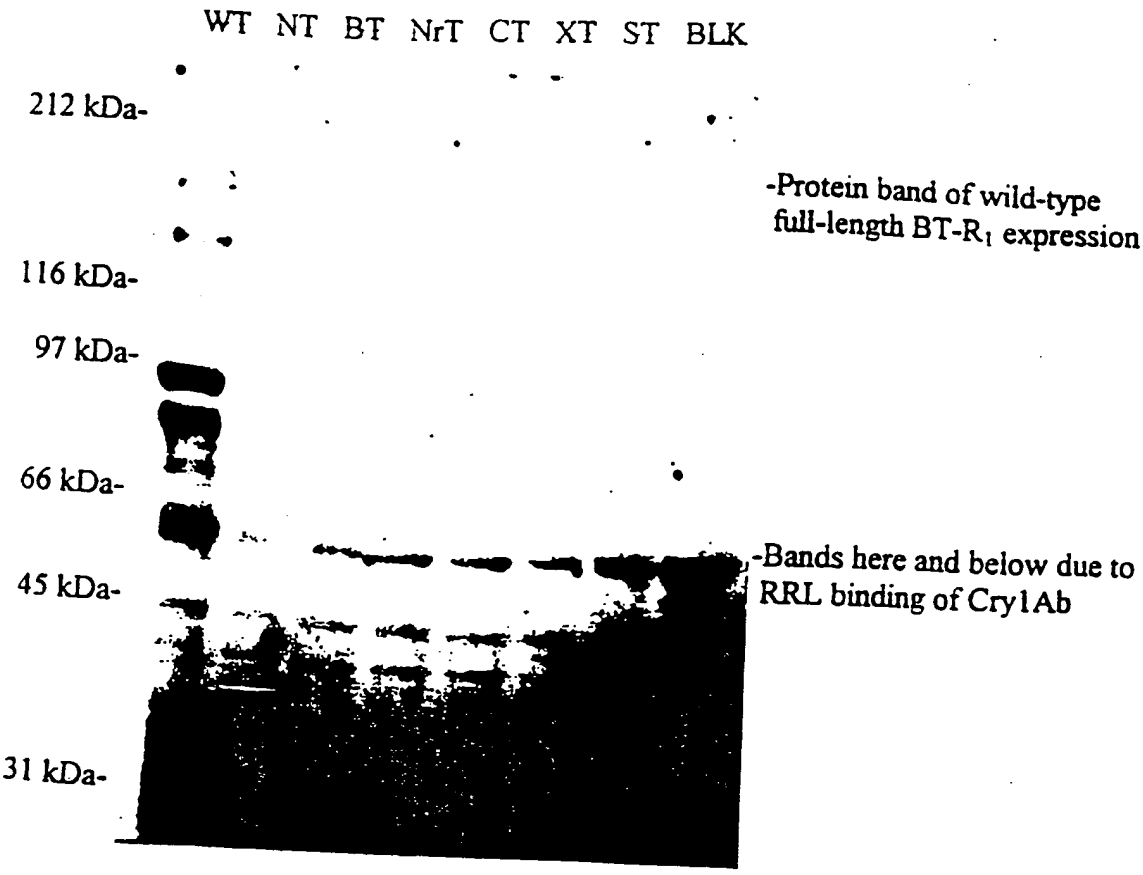

Fig. 8 Radio-blot of BT-R_1 and truncation mutants of BT-R_1 with ^{125}I-labeled Cry1Ab. WT is the wild-type full-length BT-R_1 receptor; NT is the truncation mutant resulting from NsiI digestion; BT is the mutant made with BamHI; NrT is the mutant made with NruI; CT is the mutant made with ClaI; XT is the mutant made with XhoI; ST is the mutant made with StuI; and, BLK is rabbit reticulocyte lysates containing only endogenous proteins.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 98/11868

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12N15/12 C07K14/435 C07K16/18 G01N33/566 //C07K14/325

According to International Patent Classification(IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C12N C07K G01N

Documentation searched other than minimumdocumentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 96 12964 A (UNIV WYOMING) 2 May 1996 see page 3, line 9 - line 17 see page 7 - page 8 see claims 1-14	1-10, 12-15
A		11

X	VADLAMUNDI R.K.: "Cloning and expression of a receptor for an insecticidal toxin of Bacillus thuringiensis" J. BIOL. CHEM., vol. 270, no. 10, 10 March 1995, pages 5490-5494, XP002080803 see the whole document	1-10, 12-14

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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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Date of the actual completion of theinternational search	Date of mailing of the international search report
14 October 1998	23/10/1998

Name and mailing address of the ISA	Authorized officer
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INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 98/11868

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	DORSCH J:A. & BULLA L.A.: "Determination of the specific region of BT-R1 to which the CryaAb toxin of Bacillus thuringiensis subsp. Berliner binds." FASEB JOURNAL (ABSTRACTS), vol. 11, no. 9, 31 July 1997, page A1050 XP002080380 see the whole document	11

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INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/US 98/11868

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9612964 A	02-05-1996	US 5693491 A	02-12-1997
		AU 4001595 A	15-05-1996
		CA 2200427 A	02-05-1996
		EP 0787299 A	06-08-1997
		JP 10508198 T	18-08-1998
		ZA 9508851 A	11-06-1996